HHS Public Access

Author manuscript

Food Chem Toxicol. Author manuscript; available in PMC 2024 September 01.

In silico modeling-based new alternative methods to predict drug and herb-induced liver injury: A review

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Abstract

New approach methods (NAMs) have been developed to predict a wide range of toxicities through innovative technologies. Liver injury is one of the most extensively studied endpoints due to its severity and frequency, occurring among populations that consume drugs or dietary supplements. In this review, we focus on recent developments of *in silico* modeling for liver injury prediction using deep learning and *in vitro* data based on adverse outcome pathways (AOPs). Despite these models being mainly developed using datasets generated from drug-like molecules, they were also applied to the prediction of hepatotoxicity caused by herbal products. As deep learning has achieved great success in many different fields, advanced machine learning algorithms have been actively applied to improve the accuracy of *in silico* models. Additionally, the development of liver AOPs, combined with big data in toxicology, has been valuable in developing *in silico* models with enhanced predictive performance and interpretability. Specifically, one approach involves developing structure-based models for predicting molecular initiating events of liver AOPs, while others use *in vitro* data with structure information as model inputs for making predictions. Even though liver injury remains a difficult endpoint to predict, advancements in machine learning algorithms and the expansion of *in vitro* databases with relevant biological knowledge have made a huge impact on improving *in silico* modeling for drug-induced liver injury prediction.

1. Introduction

Due to the threats from severe disease outbreaks posed to human health (Tong et al., 2022), there is an urgent need for cost-effective and time-efficient toxicity testing methods to protect public health by accelerating the development of medicine (Baker et al., 2022). Traditional toxicity testing has relied on animal models; however, this approach has its inherent drawbacks, including substantial time and costs (Van Norman, 2019), as well as limited accuracy when extrapolating results to humans (Parish et al., 2020).

New approach methods (NAMs) are animal-free methods based on innovative technologies used to assess hazardous effects of chemicals. Cost-effectiveness and time efficiency are critical factors for NAMs, but their ability to accurately predict adverse outcomes in humans is also essential. NAMs have been widely used for cosmetics (Cronin et al., 2022), where animal testing for cosmetics and their active ingredients is banned in many countries (Sreedhar et al., 2020). The use of NAMs has also been considered for the safety evaluation of pesticides and industrial chemicals (Stucki et al., 2022; van der Zalm et al., 2022). For the evaluation of pharmaceutical toxicity, *in vitro* tests for predicting organ toxicity were developed (Brecklinghaus, 2020), and NAMs for biokinetics prediction can be applied (Punt et al., 2020).

Drug-induced liver injury (DILI) has caused significant loss of time and resources in drug development projects, since DILI liability is usually identified in the late stages of clinical trials (Kaplowitz, 2005). To predict DILI in the early phases of drug development, *in vitro* tests and *in silico* models have been developed. However, the lack of standardization in *in vitro* test often leads to contradictory results (Atienzar and Nicolas, 2018) and *in silico* models have yet to achieve sufficient sensitivity identifying DILI risk (Matthews et al., 2009). Despite the substantial progress made in this area, accurately predicting DILI remains a challenging endpoint.

Liver injury is also commonly associated with the use of herbal products (Lin et al., 2019). In line with the growing consumption of herbal products (Amadi and Orisakwe, 2018), reports of herb-induced liver injury (HILI) have significantly increased in past decades (Nunes et al., 2022). Herbal supplements are generally extracted from herbs, resulting in cocktails of multiple compounds with unknown toxic effects. The interactions between compounds in these mixtures could result in synergetic effects leading to HILI, highlighting the need of effective methods predicting this type of injury.

This review focuses on *in silico* models for the prediction of hepatotoxicity associated with the use of drugs and herbal dietary supplements. The majority of *in silico* models belong to (quantitative) structure-activity relationship, or (Q)SAR, models, which predict target endpoints based solely on molecular structures. To improve their prediction accuracy, *in vitro* data have been incorporated into these models as well. Recent developments in *in vitro* assay databases and the adverse outcome pathway (AOP) model have contributed significantly to the improvement of hepatotoxicity prediction by providing additional information on compounds' mechanisms of action. Here, this review primarily concentrates

on recent and notable achievements in DILI or HILI prediction to minimize redundancy with other reviews (Vall et al., 2021).

2. Hepatotoxicity phenotypes

A wide range of (Q)SAR models have been developed to predict hepatotoxicity phenotypes, which are typically categorized based on the endpoints being predicted. Most (Q)SAR studies begin with data curation; therefore, dataset curation and the model are introduced together in this section. A list of free or commercial software for predicting hepatotoxicity and relevant endpoints is shown in Table 1.

2.1. DILI annotation

Three different data sources were used to annotate DILI risk in humans: U.S. Food and Drug Administration (FDA) labeling, clinical case reports, and literature. There is a wide range of datasets curated for DILI prediction; however, DILI annotations from different datasets were sometimes contradictory (Thakkar et al., 2018). Since there is no ground truth for DILI annotations on drugs, *in silico* model development studies sometimes go through data curation and define their own DILI annotations. Lack of a standardized DILI dataset is the major obstacle in validating a model since discrepancies among DILI annotations impose inherent prediction errors. To harmonize different DILI datasets, Thakkar et al. developed a DILI severity and toxicity (DILIst) dataset (Thakkar et al., 2020) by augmenting DILIrank (Chen et al., 2016b) with a large volume of human DILI datasets such as LiverTox (Hoofnagle, 2013), Suzuki et al. (Suzuki et al., 2010), Greene et al. (Greene et al., 2010), and Zhu et al. (Zhu and Kruhlak, 2014).

2.2. Curation of post-marketing case reports

DILI was often reported during the post-market phase, and the frequency of post-marketing reports was considered as evidence for assigning DILI labels to drugs. Zhu et al. (Zhu and Kruhlak, 2014) curated the FDA's Adverse Event Reporting System (FAERS) database to annotate drugs on four endpoints: liver damage, cholestasis, liver enzyme abnormalities, and bile duct disorders, based on the standardized terms from MeDRA (Medical Dictionary for Regulatory Activities) hierarchy. Shin et al. (Shin et al., 2020) used the frequencies of post-marketing reports on four indications (cholestasis, cirrhosis, hepatitis, and steatosis) collected from the PharmaPendium database to assign positive annotations to drugs, while the negative data was obtained from DILIrank (Chen et al., 2016b). However, no databases provided drug metabolite structures with their DILI annotations. Therefore, annotations from the parent drugs were assigned to the drug metabolites in this study. ToxSTAR is an available software product for classification of the liver injury indications for drugs and drug metabolites (Shin et al., 2022a).

2.3. DILI biomarker prediction

Since liver injury is diagnosed by serum biomarkers (Robles-Díaz et al., 2016), (Q)SAR models were developed to predict abnormal increases in these biomarkers. Rodgers et al. developed models for alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and gamma-glutamyl

transpeptidase (GGT) based on the Human Liver Adverse Effects Database (Rodgers et al., 2010).

An available software for the DILI biomarker prediction is ADMET Predictor. Liu used ADMET Predictor to predict HILI using molecular structures of natural products-derived compounds (NPCs) (Liu, 2018). In this study, only NPCs predicted to be absorbed in the gastrointestinal tract were selected, and possible metabolites from the selected NPCs were predicted. The structures of both parent compounds and their metabolites were used to predict the abnormal elevation of the biomarkers such as ALT, AST, and LDH.

3. Advances of *in silico* modeling for predicting liver toxicity

3.1. *In silico* modeling for predicting DILI in humans

Numerous (Q)SAR models for DILI prediction were developed for binary classification of DILI-positive (hepatotoxicant) or negative (no evidence of hepatotoxicity) using machine learning algorithms (Vall et al., 2021). Some DILI prediction software is publicly available, such as ProTox-II (Banerjee et al., 2018), VEGA, and Derek Nexus (Table 1). ProTox-II uses a random forest model, whereas VEGA and Derek Nexus use rule-based models. Recently, Li et al. developed DeepDILI for DILI prediction. Usually, DILI prediction models take molecular structure as an input; however, in their study the input of the deep neural network were the outputs from a set of (Q)SAR models (Li et al., 2021). Graph neural network (GNN) can be used for node classification, edge prediction, and graph classification. Molecular structure can be represented as a graph with atoms as nodes and covalent bonds as edges, and DILI prediction can be converted into a graph classification problem, where GNN predicts DILI annotation from a molecular graph.

Xu et al. used undirected graph recursive neural networks (UGRNN) for DILI prediction in which UGRNN gathers atomic information sequentially to each root atom for encoding, and then feeds encoded vectors to the output layer for prediction. In this study, multiple DILI annotation datasets were integrated to expand the sample size and improve model training (Xu et al., 2015). Similarly, Ma et al. used a multi-view graph recursive neural networks (MV-GNN) model to encode atom and bond-oriented input representation for predicting DILI risk. Since a large volume of data is required for MV-GNN to learn appropriate molecular representation, four toxicity datasets (hERG, phospholipidosis, Ames test, and mitochondrial membrane potential) were combined with DILI annotation data and multilabel training was applied. (Ma et al., 2021). These studies demonstrate the effectiveness of deep learning methods, such as UGRNN and MV-GNN, for improving DILI prediction.

Some studies applied (Q)SAR models to predict hepatotoxicity of NPCs (Kim and Nam, 2017; Li et al., 2018). In the study by Li et al., a support vector machine model with a MACCS (Molecular ACCess Systems keys) fingerprint achieved the highest accuracy, and the model was used to identify DILI-positive components in the herbal products. Kim and Nam proposed a novel fingerprint by assigning weights on each bit of PubChem fingerprint based on Bayesian probability calculated from DILI annotations. Because both studies applied (Q)SAR models derived from synthetic drug compounds for predicting HILI, there

is a potential gap in model generalization considering differences in chemical space between NPCs and synthetic drug compounds (Stratton et al., 2015). Applicability domain analysis is suggested to reduce the model's uncertainty and improve reliability of the prediction values (Weaver and Gleeson, 2008; Sutton et al., 2020).

3.2. *in silico* modeling for predicting liver toxicity in animals

Currently, *in vivo* animal studies are mandatory submissions to a regulatory agency in support of first-in-human studies. Considering animal studies' high cost and time commitment, animal-free NAMs are proposed, including *in silico* models developed from accumulated animal studies to predict *in vivo* outcomes. Mulliner et al. curated data based on the hierarchical endpoint tree, in which hepatotoxicity was branched into clinical chemistry findings or histological reports for defining hepatobiliary or hepatocellular injury. Binary classification was defined for humans based on clinical and post-marketing data, and preclinical data with doses of less than 500 mg/kg, respectively. The investigators identified the best performance model for the internal use of early preclinical safety in-silico workflow within their institutes. (Mulliner et al., 2016).

On the contrary, He et al. labeled drugs for hepatotoxicity by integrating human data (clinical data), animal experiment, and *in vitro* assay results. Thus, the model prediction is positive if evidence of hepatic injury is found in clinical studies, animal experiments, or cell-based assays (He et al., 2019). Cotterill et al. compiled hepatic steatosis datasets based on *in vivo* histology data and human clinical data retrieved from the literature, and a binary classification model was developed (Cotterill et al., 2020). Additionally, VEGA 1.2.0 provides a hepatotoxicity prediction model based on animal data (Table 1), which can predict NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level) in the liver.

4. *In silico* models for predicting hepatotoxic key events of AOPs

AOPs define series of biological events that lead to adverse outcomes, including a molecular initiating event (MIE), which is the first biological target that interacts with the molecule, and key events (KEs). AOPs could improve understanding of the systematic biological processes initiated by toxicants; thus, information defined and curated in AOPs provides a good starting point for developing *in silico* or *in vitro* NAMs (Ankley et al., 2010). The development of high-throughput screening technologies and the integration of AOPs and *in vitro* data has paved a way for developing mechanistically driven *in silico* models for DILI prediction.

Liver AOPs provide a wide range of MIEs and KEs relevant for DILI prediction. Multiple liver AOPs have been developed and are available in the AOP wiki (Table 2). The MIEs in the AOPs can be target proteins for DILI or HILI prediction. Gadaleta et al. developed an *in silico* model for hepatic steatosis prediction by compiling ToxCast *in vitro* assay data on MIEs of hepatic steatosis AOPs (Gadaleta et al., 2018). In this study, (Q)SAR models for predicting MIEs, such as PXR, LXR, AhR, NRF2, PPARα, and PPARγ, were developed and demonstrated the capability for virtually screening chemicals that can cause hepatic steatosis. Based on the key characteristics of hepatotoxicants (Rusyn et al., 2021),

we selectively discuss certain target MIEs/KEs relevant to DILI, including bile salt export pump inhibition, oxidative stress, mitochondria dysfunction, and drug bioactivations, as well as the (Q)SAR models used for predicting these targets.

4.1. Bile salt export pump (BSEP) inhibition

Cholestasis is caused by disruption in bile flow, and the BSEP is one of the hepatic transporters responsible for excreting bile acids from hepatocytes. BSEP inhibition by drugs would lead to accumulation of drugs in hepatocytes and cause cholestasis as a consequence. (Chen et al., 2016c) Many drugs causing DILI in humans have shown the inhibitory activity to the BSEP. In AOP 27 (cholestatic liver injury induced by inhibition of the BSEP), BSEP inhibition is defined as a MIE since accumulated evidence suggests that BSEP inhibition is associated with the development of DILI. Currently, several (Q)SAR models were developed to predict BSEP inhibitors and non-inhibitors. Notably, different criteria were used to label compounds as BSEP inhibitors or non-inhibitors (Kenna et al., 2018); therefore, model predictions need to be interpreted carefully based on their specific definitions for BSEP inhibitors. A BSEP inhibition model is available in admetSAR (Table 1) (Yang et al., 2019).

Accurate prediction of BSEP inhibition using molecular structure alone is a challenging task due to the complicated mechanisms involved. BSEP inhibition can occur by interfering with ATP binding (competitive inhibitors) or BSEP kinetics (non-competitive inhibitors). To improve prediction performance, (Q)SAR models were used together with pharmacophore and molecular modeling techniques. Welch et al. used pharmacophore modeling to extract significant substructures present in the inhibitors. The (Q)SAR model was developed to predict inhibition of the BSEP and multidrug resistance protein 4 (MRP 4) as DILI was linked to the inhibition of both transporters (Welch et al., 2015). Jain et al. used molecular docking and molecular dynamics for analyzing BSEP protein structures generated through the homology modeling (Jain et al., 2017). Recently, crystal structures of BSEP complexed with inhibitors at the binding pocket have been reported (Wang et al., 2022), which provided a valuable resource for molecular modeling.

4.2 Oxidative stress and glutathione depletion

Oxidative stress can be induced by reactive oxygen species (ROS) generated in the hepatocytes. It is one of the critical events for the development of liver injury due to formation of reactive intermediates through hepatic metabolism (Villanueva-Paz et al., 2021). In AOP 220 (CYP2E1 activation leading to liver cancer), cytochrome P450 2E1 (CYP2E1) is identified as an MIE that subsequently induces oxidative stress. CYP2E1 bioactivates numerous molecules and generates reactive metabolites that can cause oxidative stress.

A (Q)SAR model was developed to predict activation of the antioxidant responsive element (ARE) pathway, which help to alleviate oxidative stress (Zhang et al., 2020). In another study by Jia et al., structural alerts and ARE assay data were incorporated to predict hepatotoxicity (Jia et al., 2022). The nrf2 ARE assay was selected among 24 *in vitro* assays for its high correlation to hepatotoxicity. A (Q)SAR model was developed to predict ARE assay outcome to fill data gaps caused by the lack of testing results for certain compounds.

Twenty-seven structural alerts for oxidative stress were identified and combined with ARE assays for classifying compounds into three categories: toxic, non-toxic, or inconclusive. The combination model predicted compounds as toxic or non-toxic when structural alerts and ARE assays were both positives or negatives, and inconclusive if they did not agree with each other. This mechanistically driven model demonstrated a high correlation to hepatotoxicity (positive predictive value=0.64) and confirmed the importance of oxidative stress as a critical toxicity event leading to hepatotoxicity. ProTox-II provides a nrf2/ARE classification model (Table 1).

4.3 Mitochondrial dysfunction

As adenosine triphosphates (ATPs) are produced in mitochondria, mitochondrial damage can lead to ATP depletion, and eventually to cell death. Mitochondrial impairment has been identified as a significant key event in the development of liver injury. In AOP 273 (mitochondrial complex inhibition leading to liver injury), an inhibitor binding to one of the mitochondrial complexes from I to V is defined as an MIE for liver injury. DILI drugs and certain herbal products were frequently reported to cause mitochondrial toxicity, including free radicals generation, membrane potential loss, and mitochondrial permeability transition (Ramachandran et al., 2018).

Several (Q)SAR models were developed to predict mitochondrial toxicity based on databases including Tox21, ChEMBL, PubChem, and DrugBank (Hemmerich et al., 2020; Bringezu et al., 2021; Zhao et al., 2021). Rana et al. used mitochondrial toxicity assays and physicochemical properties of drugs to predict hepatotoxicity, cardiotoxicity, and nephrotoxicity. Compared to other organ toxicities, more hepatotoxic compounds tested positive in the mitochondrial assay. Specifically, an isolated rat liver mitochondrial inhibition assay showed a higher correlation for hepatotoxicity prediction compared to that of other assays (Rana et al., 2019). DILIsym is a physiologically-based pharmacokinetic (PBPK) model to predict biomarkers of DILI. It runs simulations through the molecular properties, physiological parameters, and mechanistically driven *in vitro* assay data. DILIsym is particularly useful for examining the importance of mitochondrial toxicity involved in development of liver injury, since some of parameters were obtained from MITOsym, an *in silico* model for predicting mitochondrial dysfunction (Lin et al., 2022). A mitochondrial toxicity classification model is available in admetSAR, and a mitochondrial membrane potential classification model in ProTox-II. (Table 1)

4.4 Drug metabolism and bioactivation

Drug metabolism increases hydrophilicity of xenobiotics to create metabolites that are more easily excreted from the body. However, reactive metabolites (RMs), which are electrophilic species that can covalently bind to proteins and DNA and leads to toxicity, can also be formed via drug metabolism. RMs were well-known to be involved in development of liver injury (Weaver et al., 2020). Chen et al. developed a logistic regression model using daily dose/Cmax, logP, and RM formation for DILI prediction. RM formation was identified as the most significant factor in the model prediction because the highest coefficient was assigned to RM formation in the model (Chen et al., 2016a). A wide range of models are available for drug metabolism prediction, such as cytochrome P450 substrate classification,

site of metabolism prediction, and drug metabolite structure prediction (Table 3). However, these models are still struggling with insufficient accuracy in drug metabolism prediction. For example, drug metabolites were usually generated through interactions with more than one drug metabolizing enzyme (DME), while the current developed models predict drug metabolism from a single DME; thus, significant gaps exist between the prediction and practice in drug metabolism.

4.5 Identification of key events based on toxicogenomic database

Understanding the mechanism of DILI is as important as accurately predicting DILI. Toxicogenomic data provide a landscape of biological perturbations caused by toxicants. These data have been used to understand DILI mechanisms and identify possible key events (Shin et al., 2022b), as well as to design human liver cell models (Lauschke, 2021). Table 4 summarizes the toxicogenomics databases that have been used to enrich our understanding of the mechanisms underlying the adverse outcomes.

Since the cost of toxicogenomic database generation is high, Chen et al. proposed ToxGAN, a generative adversarial network (GAN) using artificial intelligence, as an alternative method to generate toxicogenomic data for chemicals (Chen et al., 2022). The ToxGAN model uses molecular structure, dose, and exposure time as inputs and generates predicted toxicogenomic profiles through a deep neural network. While the data gap remains, deep learning and biological big data present new opportunities for improving the accuracy of *in silico* models for DILI prediction.

5. Integration of *in silico* and *in vitro* data

Since both *in silico* and *in vitro* models alone are insufficiently accurate for predicting DILI in humans, there has been increasing interest in the integration of these two data types. Chen et al. suggested a two-tiered approach for DILI prediction based on rule of two (RO2) and high content screening (HCS) assays. The RO2 classifies a drug as positive if a daily dose is equal to or more than 100 mg/day and its logP is greater or equal to 3. HCS would be only applied to those classified as negative by RO2, which significantly reduced the number of molecules to be screened (Chen et al., 2014). This study showed that the integration of *in vitro* data can improve hepatotoxicity prediction compared to use of the RO2 alone. Another benefit of incorporating *in vitro* assay data is its interpretability, enabling the mechanism of toxicity to be understood, while (Q)SAR models were usually "black-box" due to use of chemical descriptors together with complicated machine learning/deep learning algorithms. As *in vitro* assay data in the public domain has been increasingly accumulated through projects such as Tox21 and ToxCast, *in silico* models can be developed together with *in vitro* data to improve toxicity prediction.

Xu et al. developed multiple models for predicting organ toxicities by using chemical structure and *in vitro* assay data (Xu et al., 2020). The liver toxicity prediction model achieved the highest performance using structural information and *in vitro* assay data together. Interestingly, more *in vitro* data were used than structural data to achieve good accuracy, whereas other organ toxicity models used less *in vitro* data. Khadka et al. developed a DILI prediction model using drug properties and *in vitro* data, and relevant

KEs from liver AOPs were used to guide the selection of *in vitro* assays from Tox21 and L1000 datasets (Khadka et al., 2020). Williams et al. developed a Bayesian model using a mechanistically relevant hepatic safety *in vitro* assay together with logP and Cmax values (Williams et al., 2020). In this study, DILI occurrence at different doses was tested by modifying the exposure of the chemical through Cmax, which is one of the model variables.

The integration of *in silico* and *in vitro* data has not always been reported to improve prediction accuracy in DILI models. Ye et al. showed that DILI prediction models based on *in vitro* and structural information achieved similar prediction accuracies with the model based on structural information alone. One possible reason for this could be insufficient coverage of the biological response space by available *in vitro* data (Ye et al., 2022). In some senses, biological information is critical for achieving good prediction of DILI. Kohonen et al. developed a DILI prediction model from toxicogenomic and cytotoxicity data, assuming that transcriptional response patterns could be shared among similar hepatotoxic drugs. (Kohonen et al., 2017). The developed genomic model was reported to consistently outperform predictions generated from (Q)SAR analysis.

6. Challenges

In silico modeling for liver injury prediction has made significant progress in the past decade; however, further improvements are still needed to accurately predict liver liabilities caused by drugs and dietary supplements. Existing DILI prediction models often suffered from low sensitivity, typically 55–65%. Additionally, the general applicability of the models to other datasets is limited since the models were trained and tested on specific datasets. Recently, advancements in machine learning algorithms and expansion of *in vitro* databases have made significant impacts on *in silico* modeling for DILI prediction. However, due to the complicated mechanisms underlying the development of DILI, even the models of integrating *in silico* and *in vitro* data are still limited by insufficient prediction performance.

DILI is often reported with the administration of multiple drugs and dietary supplements. Currently, the methods covered here are not sufficient for predicting liver injury caused by mixtures of chemicals. Moreover, many DILI events are immune-mediated and cannot be easily modeled through *in silico* or *in vitro* systems. Growing evidence has suggested that DILI results from interactions between drug properties, as well as environmental and host factors (Chen et al., 2015). Variations in the human leukocyte antigen genes are also reported to be associated with DILI susceptibility (Daly, 2023). These factors, in addition to individual differences in DMEs and hepatic transporter expression, should be considered in order to develop an improved model for DILI prediction in humans.

Data availability

None

Acknowledgements

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Table 1.

Selected liist of software for hepatotoxicity or relevant endpoint prediction

Name	Endpoint	License type	Prediction models available at
ADMET predictor	Elevation of DILI biomarkers (ALP, AST, LDH)	Commercial software (GUI* program)	
Derek Nexus	Severity of DILI	Commercial software (GUI* program)	
VEGA	Hepatotoxicity (Positive/Unknown/Negative)	Freeware (GUI program)	https://www.vegahub.eu/portfolio-item/vega-qsar/
	Liver NOAEL and LOAEL		
	Hepatic steatosis MIEs (PXR/PPARα/PPARγ/NRF2)		
ProTox–II	Hepatotoxicity classification (Positive/Negative)	Freeware (Web program)	https://tox-new.charite.de/protox_II/
	Nrf2/ARE classification		
	Mitochondrial membrane potential classification		
ToxSTAR	Binary classification of DILI indications (Cholestasis, Cirrhosis, Hepatitis, and Steatosis)	Freeware (Web program)	https://toxstar.kitox.re.kr/
admetSAR	Hepatotoxicity classification	Freeware (Web program)	http://lmmd.ecust.edu.cn/admetsar2
	BSEP inhibition		
	Mitochondrial toxicity		

*
GUI: Graphical User Interface

Table 2.

Selected list of liver AOPs from AOP wiki (accessed on 21 Mar 2023)

AOP ID	Title	Molecular initiating events (MIEs)
27	Cholestatic liver injury induced by inhibition of the bile salt export pump (ABCB11)	Bile salt export pump inhibition (ABCB11)
34	LXR activation leading to hepatic steatosis	LXR activation
36	Peroxisomal Fatty Acid Beta-Oxidation inhibition leading to steatosis	PPAR promoter demethylation
57	AhR activation leading to hepatic steatosis	PPAR-α, β, γ activation decrease
		AhR activation
58	CAR suppression leading to hepatic steatosis	CAR suppression
		PPAR-α inhibition
		LXR activation
		PPAR promoter demethylation
59	HNF4 α suppression leading to hepatic steatosis	HNF4 α suppression
60	PXR activation leading to hepatic steatosis	PXR/SXR activation
61	NFE2L2/FXR activation leading to hepatic steatosis	NRF2 activation
		NR1H4 activation
62	AKT2 activation leading to hepatic steatosis	Systemic inflammation leading to hepatic steatosis
220	CYP2E1 activation leading to liver cancer	Activation of CYP2E1
232	NFE2/Nrf2 repression to steatosis	NFE2/Nrf2 repression
273	Mitochondrial complex inhibition leading to liver injury	Inhibition of any mitochondrial complexes (I, II, III, IV, or V)
278	IKK complex inhibition leading to liver injury	IKK complex inhibition
317	Glucocorticoid receptor activation leading to hepatic steatosis	Glucocorticoid receptor activation

Table 3.

Selected list of software for drug metabolism prediction

Name	Endpoint	License type	Prediction models available at
ACD/Percepta	CYP450 inhibitor/substrate	Commercial (GUI program)	
	P-gP inhibitor/substrate		
	Phase I site of metabolism		
ADMET predictor	Phase I site of metabolism (Metabolite structure prediction)	Commercial (GUI program)	
Meteor Nexus	Metabolic fate prediction	Commercial (GUI program)	
PreADMET	CYP450 inhibitor/substrate	Freeware (Web program)	https://preadmet.webservice.bmdrc.org/
	P-gP inhibitor		
	Phase I site of metabolism		
PreMetabo	Phase I & II drug metabolism inhibitor/substrate	Freeware (Web program)	https://premetabo.webservice.bmdrc.org/
	P-gP substrate		
admetSAR	Phase I, II, and transporter inhibitor/substrate	Freeware (Web program)	http://lmmd.ecust.edu.cn/admetsar2
SMARTcyp	CYP450 site of metabolism	Freeware (Web program)	https://smartcyp.sund.ku.dk/mol_to_som
Xenosite	Site of metabolism (Epoxidation, Quinonation, Reactivity, Phase I, N-dealkylation, UGT conjugation)	Freeware (Web program)	https://xenosite.org/

Table 4.

Selected list of toxicogenomics database (accessed on 6th July)

Abbreviations	Descriptions	Available at
BioPlanet	Integration of human pathways with the healthy and disease state annotations and targets.	https://tripod.nih.gov/bioplanet/
CHemDIS	Chemical-disease inference system is deployed with interfaces for enrichment analysis for functions, pathways and diseases to identify chemicals with potential risks.	http://cwtung.kmu.edu.tw/chemdis
CTD	Comparative Toxicogenomics Database provides manually-curated chemical-gene/protein interactions, chemical-disease and gene-disease relationships curated manually.	https://ctdbase.org/
DisGeNET	DisGeNET is a platform to integrate information on human disease-associated genes and variants.	https://www.disgenet.org/
DrugMatrix	Toxicogenomic reference resources. Data is reported through ToxFX. It is no longer supported by National Toxicology Program; however, source code of DrugMatrix and ToxFX will be available in Github	https://ntp.niehs.nih.gov/data/drugmatrix
LINCS	The Library of Integrated Network-Based Cellular Signatures contains a variety of data based on the data level concept in the Cancer Genome Atlas.	https://lincsproject.org/
MSigDB	Molecular Signatures Database provides annotated gene sets for [*]GSEA software	https://www.gsea-msigdb.org/gsea/msigdb
Reactome	Reactome is a biological pathway database manually curated and peer-reviewed.	https://reactome.org/
TG-GATEs	Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System is developed from the Japanese Toxicogenomics Project Consortium from 170 compounds.	https://www.toxicodb.ca/datasets/1

[*] GSEA: Gene Set Enrichment Analysis