AOP-Liver™

iS Tox Platform™
Machine-learning models for a new toxicology

Report

Client:
Altox

Username:
Tiago

Study Number:
AOPLiver_Compound8_

Date:
2019/06/19 - 15:29:34

Program Version: 1.8

Molecular Query

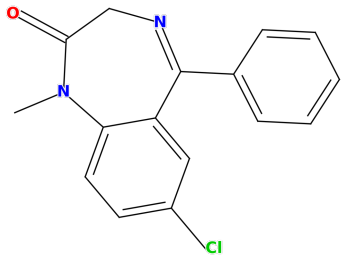
Name:
Compound 8

CAS:
NA

SMILES:
CN1c2ccc(Cl)cc2C(=NCC1=O)c1ccccc1

logK$_{ow}$:
3.15

logD:
2.75



Model Summary

AOP-Liver™ (Adverse Outcome Pathway-based model for Hepatotoxicity) is a computational tool developed by Altox using knowledge derived from a combination of mechanisms, key events, and outcomes data related to hepatotoxicity assessment.

This model integrates *in vitro* and *in vivo* data in a targeted and predictive framework that simulates an Integrated Approach to Testing and Assessment (IATA) for hepatotoxicity. The model uses a combination of structural alerts and artificial intelligence (AI) models to predict responses in different biological organisation levels: Agonists of the Antioxidant Response Element (ARE), Agonists of the Aryl Hydrocarbon Receptor (AhR), cytotoxicity assays using hepatocellular carcinoma (HepG2) cells, and in vivo Non-Rodent, Rodent and Human Drug-Induced Liver Injury (DILI). An AOP-based prediction for the outcome hepatotoxicity is obtained by integration of the individual models, balanced by adjustments and weight of evidence assessment.

To assess both direct and indirect potential Drug-Induced Liver Injury (DILI), this tool predicts the potential for metabolic activation using known Phase I reactions. AOP-Liver integrates alerts, expert system, statistical and machine learning-models to support regulatory decision-making and integrated testing strategies.

This tool is powered by 154 Structural Alerts for Drug-Induced Liver Injury (DILI). The datasets were rigorously curated with results for ARE assay, AhR assay, HepG2 assay, and Non-Rodent, Rodent and Human Drug-Induced Liver Injury (DILI), containing 5467, 6316, 4616, 880, 964 and 3268 structures, respectively.

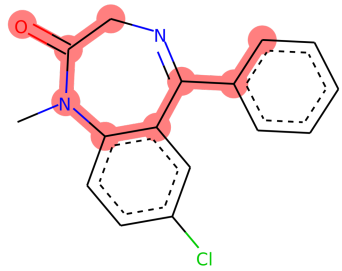
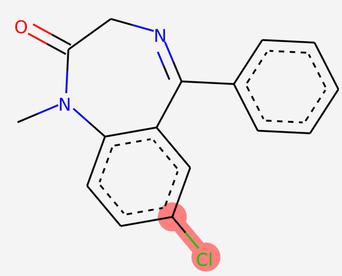
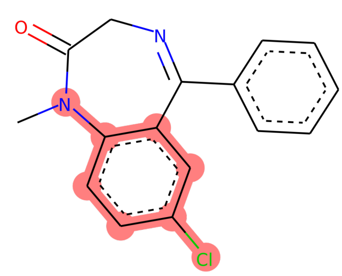
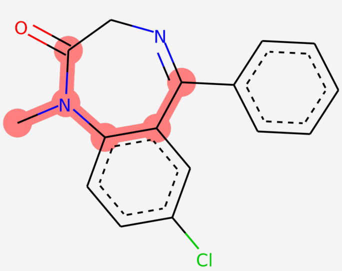
The final result is provided in a graphical plot visually summarising all the individual predictions, the applicability domain (AD) confidence levels, with a decision for the outcome.

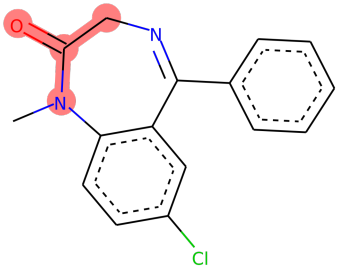
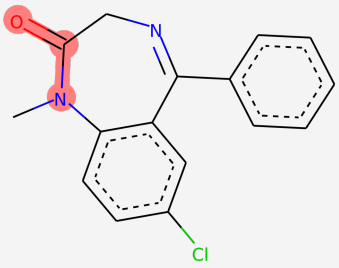
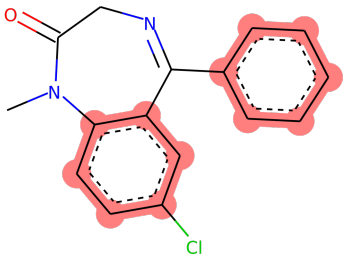
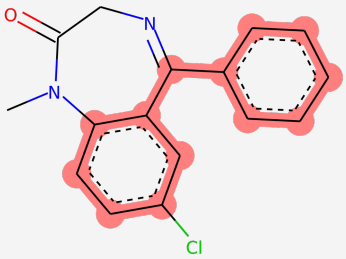
Structural Alert Analysis

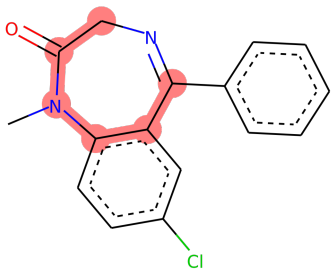
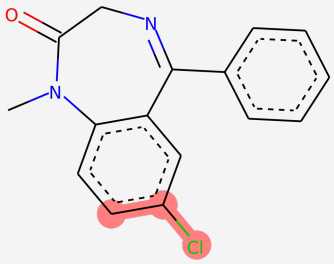
The result below is based on an analysis of fragments assigned to be hepatotoxic (*in vitro* and *in vivo* induced hepatotoxicity alerts). When possible, the alerts can provide a mechanistic basis of the predicted hepatotoxicity of the molecule or insights for interpretation of the mechanism based on theories and knowledge of toxicity mechanisms (OECD Principle 5).

Result: (+) Positive

Alerts were found in the molecule. The results are in the table below and a description is provided at the end of the report.

Category	Alert	Alert ID	References
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Lombardo A.; Manganaro A.; Benfenati E. A New Structure-Activity Relationship (SAR) Model for Predicting Drug-Induced Liver Injury Based on Statistical and Expert-Based Structural Alerts. Front. Pharmacol. 2016 7 442.
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Lombardo A.; Manganaro A.; Benfenati E. A New Structure-Activity Relationship (SAR) Model for Predicting Drug-Induced Liver Injury Based on Statistical and Expert-Based Structural Alerts. Front. Pharmacol. 2016 7 442.
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Lombardo A.; Manganaro A.; Benfenati E. A New Structure-Activity Relationship (SAR) Model for Predicting Drug-Induced Liver Injury Based on Statistical and Expert-Based Structural Alerts. Front. Pharmacol. 2016 7 442.
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Lombardo A.; Manganaro A.; Benfenati E. A New Structure-Activity Relationship (SAR) Model for Predicting Drug-Induced Liver Injury Based on Statistical and Expert-Based Structural Alerts. Front. Pharmacol. 2016 7 442.

Category	Alert	Alert ID	References
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Lombardo A.; Manganaro A.; Benfenati E. A New Structure-Activity Relationship (SAR) Model for Predicting Drug-Induced Liver Injury Based on Statistical and Expert-Based Structural Alerts. Front. Pharmacol. 2016 7 442.
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Lombardo A.; Manganaro A.; Benfenati E. A New Structure-Activity Relationship (SAR) Model for Predicting Drug-Induced Liver Injury Based on Statistical and Expert-Based Structural Alerts. Front. Pharmacol. 2016 7 442.
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Gadaleta D.; Lombardo A.; Nicolotti O.; Benfenati E. Identification of structural alerts for liver and kidney toxicity using repeated dose toxicity data. Chem. Cent. J. 2015 9 62.
Liver toxicity using repeated dose toxicity		Positive (+) (Potential Hepatotoxic)	Pizzo F.; Gadaleta D.; Lombardo A.; Nicolotti O.; Benfenati E. Identification of structural alerts for liver and kidney toxicity using repeated dose toxicity data. Chem. Cent. J. 2015 9 62.

Category	Alert	Alert ID	References
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Zhang C.; Cheng F.; Li W.; Liu G.; Lee P. W.; Tang Y. In silico Prediction of Drug Induced Liver Toxicity Using Substructure Pattern Recognition Method. Mol. Inform. 2016 35 136–44.
Drug-Induced Liver Injuries (DILIs)		Positive (+) (Potential Hepatotoxic)	Zhang C.; Cheng F.; Li W.; Liu G.; Lee P. W.; Tang Y. In silico Prediction of Drug Induced Liver Toxicity Using Substructure Pattern Recognition Method. Mol. Inform. 2016 35 136–44.

Machine Learning Models

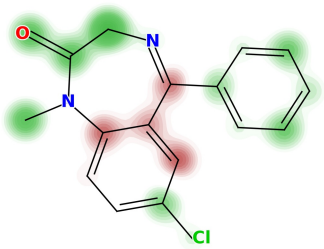
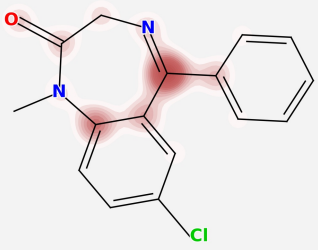
The individual predictions below were obtained by Artificial Neural Networks (ANN) covering the AOP key events and outcomes:

MIE (Molecular Initiating Event):	Reactivity, Activation of biochemical pathways such as chemicals that stimulate oxidative stress (Antioxidant Response Element, ARE) and, Aryl Hydrocarbon Receptor (AHR) activation is thought to lead to multiple adverse outcomes including hepatic steatosis.
Key event 2 (KE2):	Cell viability by human hepatocellular carcinoma cells are used to assess compound induced hepatotoxicity (HepG2)
Adverse Outcome:	Hepatotoxicity

The ANN assign a category active (+) or inactive (-) for the ARE and AhR. For the human hepatocellular carcinoma (HepG2) cells the ANN assign a potency and category strong toxicity (++), weak-moderate toxicity (+) or nontoxic (-). For the Non-Rodent, Rodent and Human for Drug-Induced Liver Injury (DILI) endpoints the ANN assign a potency category hepatotoxic (+) or non-hepatotoxic (-). To ensure transparency in the description of the deep learning model (an unambiguous algorithm - OECD Principle 2), more detailed information about each model is presented below. The STR (Structure-Toxicity Relationship) Probability Mapping indicates the fragments more related to the absence/decrease of toxicity (green) or presence/increase (red) as well, useful for hypotheses and mechanistic interpretations (OECD Principle 5).

STR Contribution Map

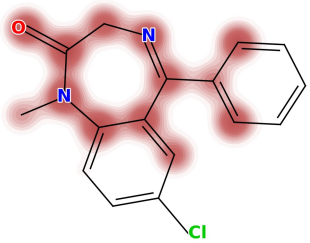
Increase Activity (+) Decrease Activity (-)

Assay/Event	Predicted class (Confidence)	STR Contribution Mapping
Agonists of Antioxidant Response Element (ARE) Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Inactive (-) (69.0%)	
Agonists of Aryl Hydrocarbon Receptor (AhR) Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Active (+) (89.2%)	

The deep learning model has components with multiple hidden layers that can learn increasingly abstract representations of the chemical hybrid descriptors. The chemical hybrid descriptor is composed by ECFP6 fingerprint and physicochemical properties [molecular weight (MW), the topological polar surface area (TPSA), octanol-water partition coefficient for neutral compounds ($logK_{ow}$) or at different pH states - logD]. Then, every 4 subsequent layers learn more complex representations. Finally, the last layer can evaluate the DILI of the given compound.

STR Contribution Map

Increase Toxicity (+) Decrease Toxicity (-)

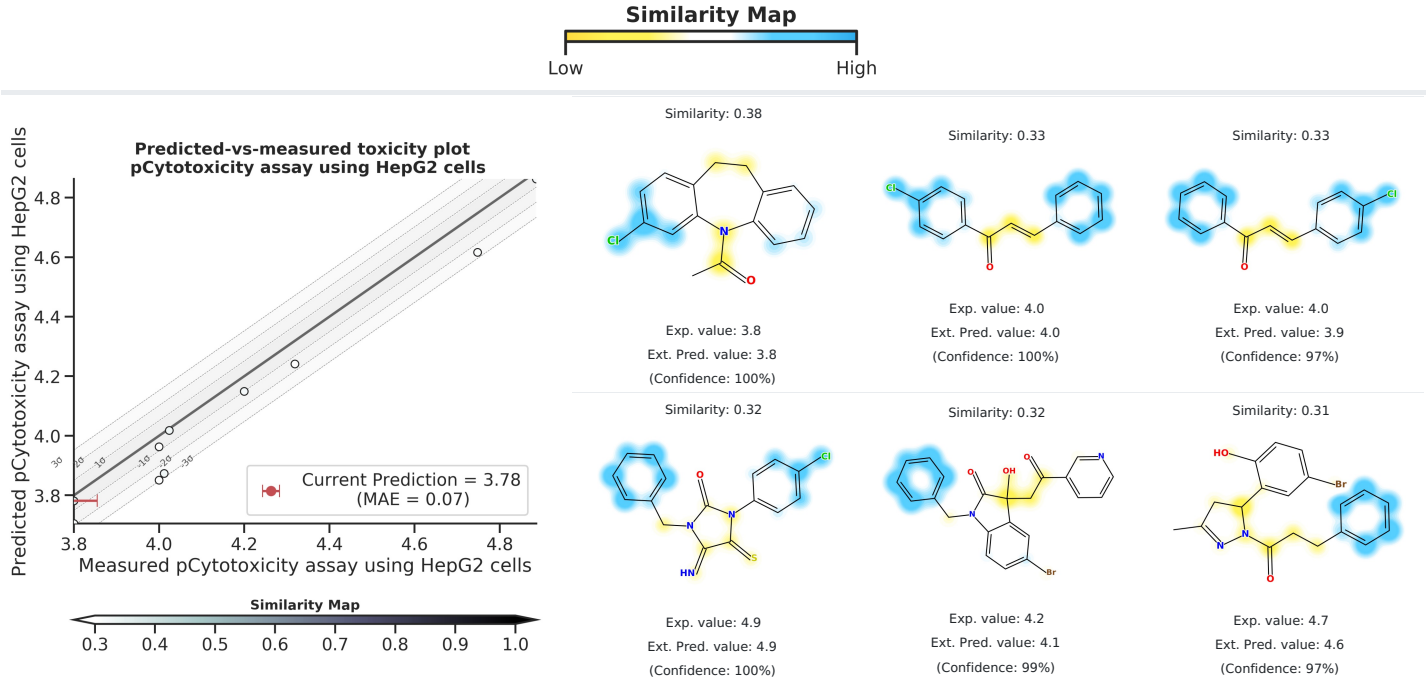
Predicted Outcome/Assay	Predicted class (Confidence)	STR Contribution Mapping
Cytotoxicity assay using HepG2 cells Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Nontoxic (-) 47.1 mg/L 165.3 µM (98.0%)	

Prediction Confidence (Based on the most similar molecules)

With appropriate measures of goodness-of-fit, robustness, and predictivity (OECD Principle 4). The graph below plots measured versus predicted acute oral toxicity values for each most similar compound and, the mean absolute error (MAE) for each prediction.

Experimental vs predicted toxicity values

for the six most similar molecules (Dice) with the prediction confidence level



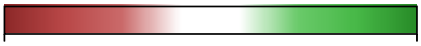
To assess the confidence of the AOP-Liver™ predictions, after of to assess the applicability domain of the model by the Visual applicability domain (AD) Inspection®, is recommended assess the model performance by the predicted-vs-measured toxicity plot and confidence level for the most similar molecules.

To assess the chemical space of the prediction and its mean absolute error (MAE), this section presents the model performance for the most similar molecules (Dice) during 5-Fold external validation, also, for the six most similar molecules (Dice) with the confidence level side-by-side to each drug-induced hepatotoxicity prediction. If the predicted value matches the experimental values for the test set chemicals, the model has a low MAE and greater confidence.

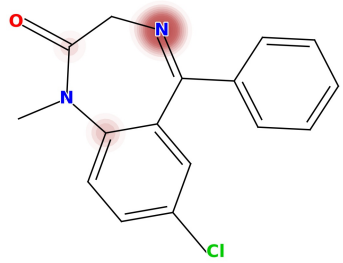
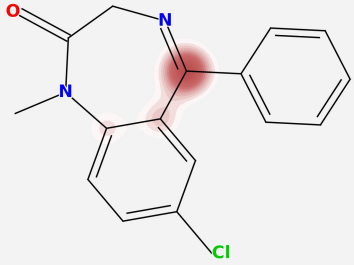
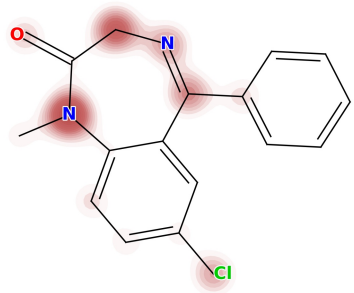
Detailed data about the dataset of chemicals, including endpoint and descriptor values; derivation of the descriptors; test and training sets; removed outliers; statistical parameters and others are available in the QMRF (QSAR Model Reporting Format) report under a confidentiality agreement. Altox Ltda assures scientific integrity of the data.

The Deep learning model has components with multiple hidden layers that can learn increasingly abstract representations of the chemical hybrid descriptors. The chemical hybrid descriptor. Then, every 4 subsequent layers learn more complex representations. Finally, the last layer can evaluate the hepatotoxicity prediction values of the given compound.

STR Contribution Map

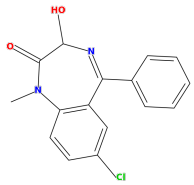
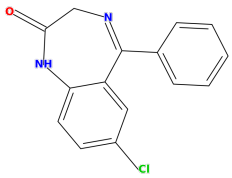
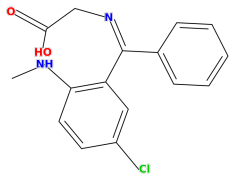
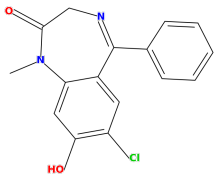


Increase Hepatotoxicity (+) Decrease Hepatotoxicity (-)

Assay/Event	Predicted class (Confidence)	STR Contribution Mapping
Non-Rodent Drug-Induced Liver Injury (DILI) Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Hepatotoxic (+) (84.4%)	
Rodent Drug-Induced Liver Injury (DILI) Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Hepatotoxic (+) (97.6%)	
Human Drug-Induced Liver Injury (DILI) Deep Learning categorical model implemented with hybrid descriptors (ECFP6 fingerprint and physicochemical properties: MW, TPSA, $logK_{ow}$, logD)	Hepatotoxic (+) (67.5%)	

Metabolism prediction and potential for hepatotoxicity

To assess both direct and indirect hepatotoxicity, this module can be used to identify potential hepatotoxic compounds which required some type of metabolism to an active metabolite (metabolic activation by known Phase I reactions) before initiation of the key event 1 (KE1) in a hepatotoxicity assessment AOP (OECD Principle 5).

Metabolite (predicted structure)				
SMILES	CN1C(=O)C(O)N=C(c2ccccc2)c2cc(Cl)ccc21	O=C1CN=C(c2ccccc2)c2cc(Cl)ccc2N1	CNc1ccc(Cl)cc1C(=NCC(=O)O)c1ccccc1	CN1C(=O)CN=C(c2ccccc2)c2cc(Cl)c(O)cc21
Reaction Rule	Aliphatic Hydroxylation	N-Demethylation	Hydrolysis	Aromatic Hydroxylation
Metabolite Score	42.1 %	41.8 %	9.6 %	6.1 %
AOP-based Prediction	**Non-hepatotoxic (-)**	**Hepatotoxic (+)**	**Hepatotoxic (+)**	**Hepatotoxic (+)**

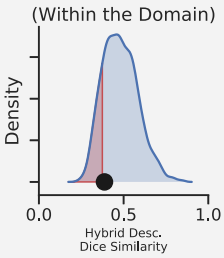
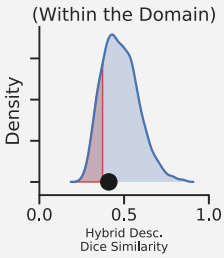
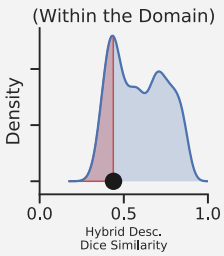
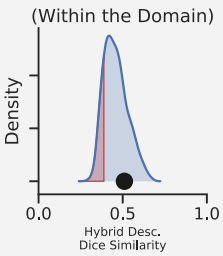
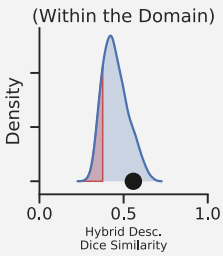
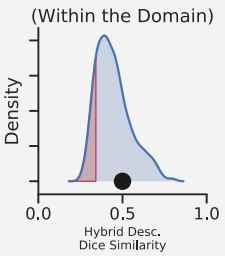
The Expert Knowledge algorithm is based on a broad range of metabolic phase 1 reactions reported in the human Metabolite Database[3]. The reactions found for the parent compound is presented in the column "reaction rule". The "metabolite score" is an empirical probability score assigned to each rule representing the fraction of correctly predicted metabolites in the training set to improve the predictivity. Evaluation of the rule-based predictions demonstrated a significant enrichment of true metabolites in the top list, e.g. the algorithm was able to identify 84 % of the cytochrome P450 specific metabolites and moreover, 66 % of the metabolites were in the top three predicted phase 1 metabolites.

Visual AD Inspection®

The applicability domain (AD) is defined by the chemical structure space and the toxicological response encoded by the developed model, to make new predictions with a given reliability (a defined domain of applicability - OECD Principle 3).[2] Our visual AS Inspection® is used to establish the scope and limitations of the models. Basically, new chemicals must be reasonably similar to training set compounds or a prediction cannot be accepted.

Our visual AD inspection is represented by a density plot of the average fingerprint-dice similarity for the k-nearest neighbors of each compound during the 5-Fold external validation. The chemical structure is represented by a hybrid descriptor composed by ECFP6 fingerprint and physicochemical measurements: molecular weight (MW), topological polar surface area (TPSA), octanol-water partition coefficient for neutral compounds ($logK_{ow}$) or at different pH states (logD). At the visual AD inspection, the black circle represents the evaluated compound, the highlighted red area represents the restricted similarity region, and the blue region is the allowed similarity of the chemical space to predict new compounds.

Even though a well-designed AD helps the user to assess the reliability of predictions made by the model, it should not automatically be assumed that all predictions within the defined AD are necessarily reliable.

Agonists of Antioxidant Response Element (ARE)	Agonists of Aryl Hydrocarbon Receptor (AhR)	Cytotoxicity assay using HepG2 cells	Non-Rodent Drug-Induced Liver Injury (DILI)	Rodent Drug-Induced Liver Injury (DILI)	Human Drug-Induced Liver Injury (DILI)
					

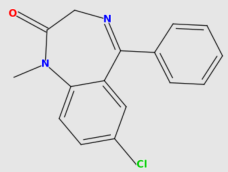
Results

The results are presented below; positive (red) and/or negative (green) predictions are presented with applicability domain (AD) and confidence level (internal goodness-of-fit and robustness, OECD principle 4) for each AOP key event and outcome prediction:

MIE (Molecular Initiating Event):	Reactivity, Activation of biochemical pathways such as chemicals that stimulate oxidative stress (Antioxidant Response Element, ARE) and, Aryl Hydrocarbon Receptor (AHR) activation is thought to lead to multiple adverse outcomes including hepatic steatosis.
Key event 2 (KE2):	Cell viability by human hepatocellular carcinoma cells are used to assess compound induced hepatotoxicity (HepG2)
Adverse Outcome:	Hepatotoxicity

An AOP-based final prediction integrating all individual key events and outcomes is provided with external predictivity (OECD principle 4), balanced by key event relationships, adjustments, and weight-of-evidence assesstment. For each individual model is attributed a weight is assigned based in its confidence level.

Chemical Structure & Predicted Properties



Predicted Physical-Chemical Properties:

logK$_{ow}$: 3.15
logD: 2.75

Predicted Molecular Initiating Event (MIE)

Structural Alerts
Positive (+)

ARE
Inactive (-)
AD: Within
Confidence: 69.0%

AhR
Active (+)
AD: Within
Confidence: 89.2%

Predicted Cellular Response (KE2)

HepG2
Nontoxic (-)
AD: Within **Confidence:** 98.0%

Predicted Adverse Outcome

Non-Rodent DILI
Hepatotoxic (+)
AD: Within
Confidence: 84.4%

Rodent DILI
Hepatotoxic (+)
AD: Within
Confidence: 97.6%

Human DILI
Hepatotoxic (+)
AD: Within
Confidence: 67.5%

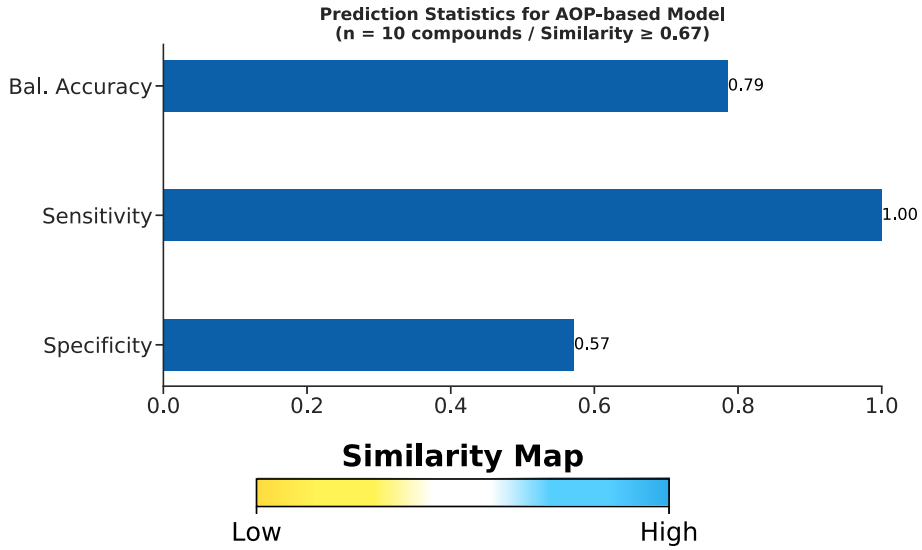
AOP-based Prediction

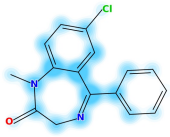
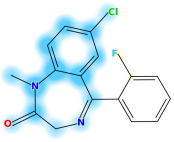
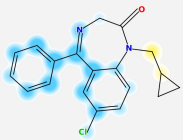
Hepatotoxic (+)

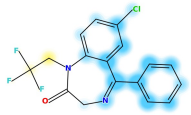
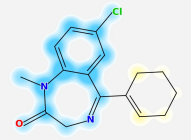
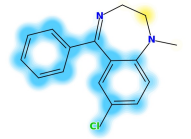
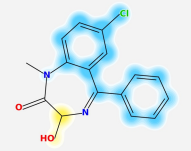
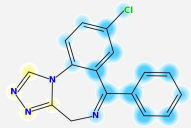
Concordance between KEs: 68.5%
Confidence level (external validation): 100.0%

Additional Information

The Hybrid descriptor Dice similarity was used to improve the deep learning confidence by interpolating the confidence equalized by the compound similarity criteria obtained from the dataset chemical space. This helps to improve the *in silico* toxicological model to reduce the false positive and negative error.



Molecule (Similarity)	Alerts	ARE Prediction	AhR Prediction	HepG2 Prediction	Non-Rodent DILI Prediction	Rodent DILI Prediction	Human DILI Prediction	Exp. Data	AOP-based Prediction (Confidence)
 (1.0)	Positive (+)	Inactive (-)	Active (+)	Weak-moderate Toxicity (+) (41.1 µM)	Hepatotoxic (+)	Hepatotoxic (+)	Hepatotoxic (+)	**Hepatotoxic (+)**	**Hepatotoxic (+) (75.8%)**
 (0.78)	Positive (+)	Active (+)	Active (+)	Nontoxic (-) (86.1 µM)	Hepatotoxic (+)	Hepatotoxic (+)	Non-hepatotoxic (-)	**Non-Hepatotoxic (-)**	**Hepatotoxic (+) (56.8%)**
 (0.76)	Positive (+)	Inactive (-)	Active (+)	Nontoxic (-) (88.1 µM)	Hepatotoxic (+)	Hepatotoxic (+)	Non-hepatotoxic (-)	**Non-Hepatotoxic (-)**	**Hepatotoxic (+) (54.3%)**
 (0.74)	Positive (+)	Active (+)	Active (+)	Weak-moderate Toxicity (+) (11.3 µM)	Non-hepatotoxic (-)	Hepatotoxic (+)	Non-hepatotoxic (-)	**Non-Hepatotoxic (-)**	**Non-Hepatotoxic (-) (52.7%)**

Molecule (Similarity)	Alerts	ARE Prediction	AhR Prediction	HepG2 Prediction	Non-Rodent DILI Prediction	Rodent DILI Prediction	Human DILI Prediction	Exp. Data	AOP-based Prediction (Confidence)
 (0.74)	Positive (+)	Active (+)	Active (+)	Weak-moderate Toxicity (+) (3.4 µM)	Non-hepatotoxic (-)	Hepatotoxic (+)	Non-hepatotoxic (-)	**Non-Hepatotoxic (-)**	**Non-Hepatotoxic (-) (51.4%)**
 (0.72)	Positive (+)	Inactive (-)	Active (+)	Weak-moderate Toxicity (+) (76.7 µM)	Non-hepatotoxic (-)	Hepatotoxic (+)	Hepatotoxic (+)	**Hepatotoxic (+)**	**Hepatotoxic (+) (69.1%)**
 (0.7)	Positive (+)	Active (+)	Active (+)	Weak-moderate Toxicity (+) (25.1 µM)	Non-hepatotoxic (-)	Hepatotoxic (+)	Non-hepatotoxic (-)	**Non-Hepatotoxic (-)**	**Non-Hepatotoxic (-) (51.9%)**
 (0.69)	Positive (+)	Active (+)	Active (+)	Weak-moderate Toxicity (+) (63.5 µM)	Non-hepatotoxic (-)	Hepatotoxic (+)	Non-hepatotoxic (-)	**Non-Hepatotoxic (-)**	**Non-Hepatotoxic (-) (50.0%)**
 (0.68)	Positive (+)	Inactive (-)	Active (+)	Weak-moderate Toxicity (+) (53.3 µM)	Hepatotoxic (+)	Non-hepatotoxic (-)	Hepatotoxic (+)	**Hepatotoxic (+)**	**Hepatotoxic (+) (72.0%)**
 (0.67)	Positive (+)	Active (+)	Active (+)	Weak-moderate Toxicity (+) (67.3 µM)	Hepatotoxic (+)	Hepatotoxic (+)	Non-hepatotoxic (-)	**Non-Hepatotoxic (-)**	**Hepatotoxic (+) (50.7%)**