Web services for *in silico* toxicity prediction in preclinical drug development

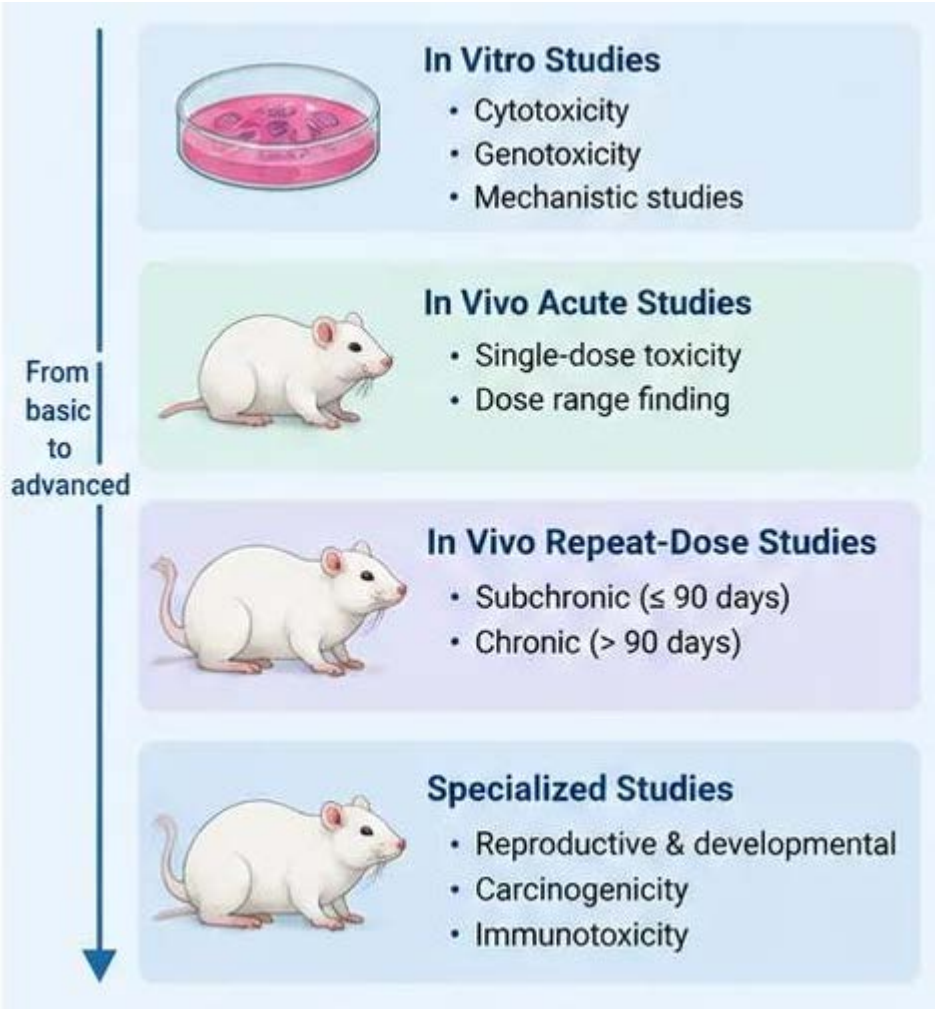
Alexey Lagunin

Institute of Biomedical Chemistry, Moscow, Russia;
Pirogov Russian National Research Medical University, Moscow, Russia.

Regulatory Framework for Preclinical Toxicology

Global Standards for Safety Assessment

- **International Consensus (ICH):**
  - **ICH M3(R2):** Guiding framework for preclinical safety data required before human clinical trials.
  - **ICH S4:** Standards for chronic toxicity evaluation in animal models.
- **United States (FDA):**
  - **21 CFR Part 58:** Federal regulation establishing strict **GLP** (Good Laboratory Practice) for non-clinical studies.
- **European Union (EMA):**
  - **Directives 2004/10/EC & 2004/9/EC:** EU legislation enforcing GLP principles and inspections.
- **Russia & EAEU:**
  - **EAEU Collegium Decision No. 81:** Unified Rules of Good Laboratory Practice, harmonized with global OECD/GLP standards.



Importance in the Drug Discovery Pipeline

Strategic Impact on Safety & Translation

- **Human Safety Guarantee:**
  Identifies toxicity profiles (acute, subchronic, chronic) before the first-in-human (FIH) dose.

- **Therapeutic Window & NOAEL:**
  Determines the **No Observed Adverse Effect Level (NOAEL)** to calculate safe starting doses for Phase I trials.

- **Mutual Acceptance of Data (MAD):**
  Conducting studies under **ICH/GLP** enables simultaneous submission to FDA, EMA, and Russia's MoH without duplicating animal tests.

- **Ethics & 3R Concept:**
  Modern regulations strictly enforce the **3R concept** (*Replacement, Reduction, Refinement*), paving the way for AI and *in silico* integration.



AI Integration into Regulatory Toxicology

The Shift Toward Validated In Silico Methods

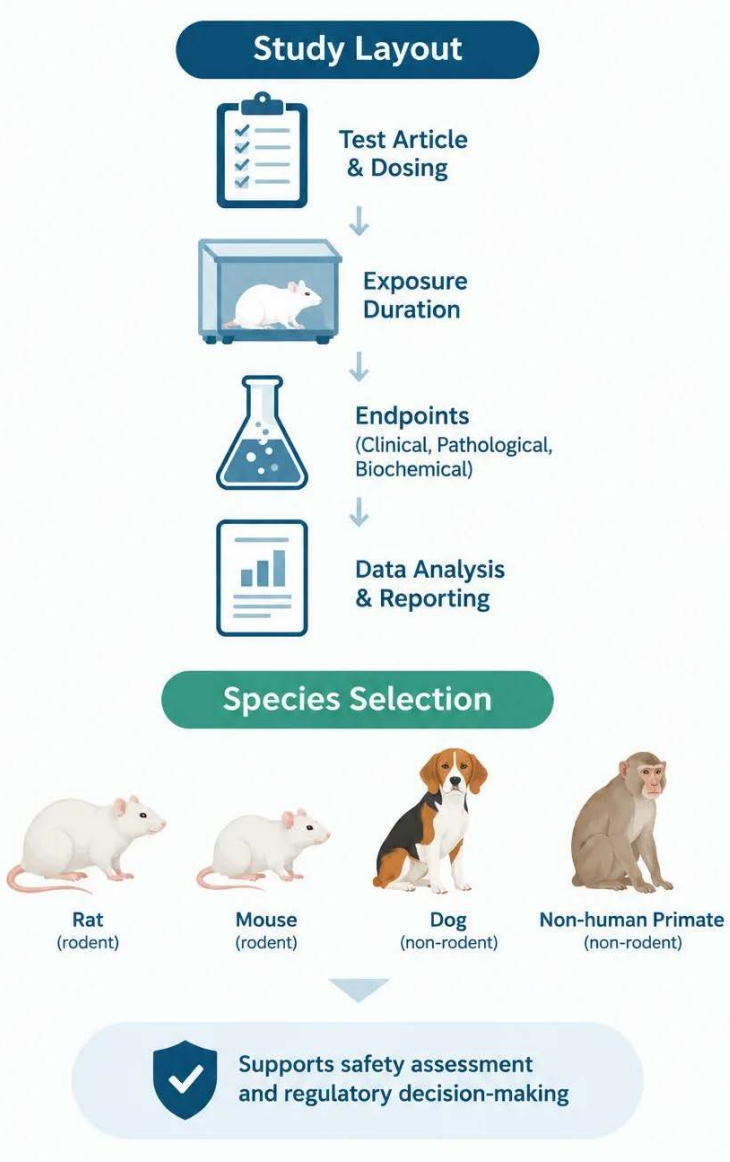
- **ICH M7 Official Recognition:**
  - The use of **two complementary (Q)SAR AI models** (rule-based and statistical) to predict genotoxic impurities, bypassing *in vivo* testing if safe.
- **FDA & EMA Roadmaps:**
  - *FDA Predictive Toxicology Roadmap:* Strategic integration of AI/ML to replace traditional animal screening.
  - *EMA Regulatory Science Strategy:* Establishing frameworks for validating computer models in Common Technical Documents (CTD).
- **Lead Optimization Filters:**
  - ML/AI models to predict **ADMET** properties, clearance rates, and off-target risks before chemical synthesis.
- **Virtual Control Groups:**
  - Using historical GLP data to reduce the number of live animal controls (3R concept).



Experimental Design: Study Layout & Species Selection

Core Requirements for General Toxicity Studies

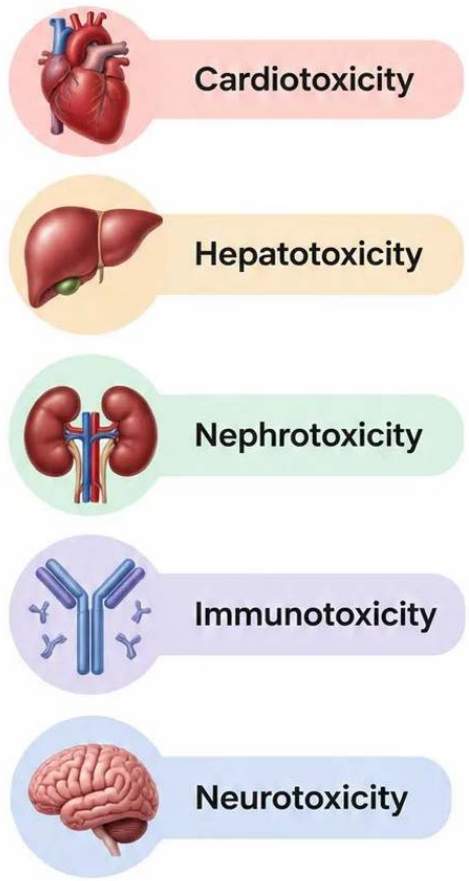
- **Two Mammalian Species Rule:**
  - Studies must include **one rodent** (rat/mouse) and **one non-rodent** (dog, minipig, or non-human primate) to account for metabolic variations.
- **Core Battery of Tests:**
  - *Acute Toxicity:* Evaluation of single-dose effect to find Maximum Tolerated Dose (MTD).
  - *Repeat-Dose Toxicity:* Daily administration lasting from 2 weeks to 6–9 months.
  - *Genotoxicity:* In vitro Ames test coupled with in vivo chromosomal aberration assays.
- **Dose Groups:**
  - Minimum of **three dose levels** plus a vehicle control group. The high dose must induce clear but non-lethal toxicity to reveal target organs.



Organ-Specific Toxicity

Evaluation of Critical Physiological Systems

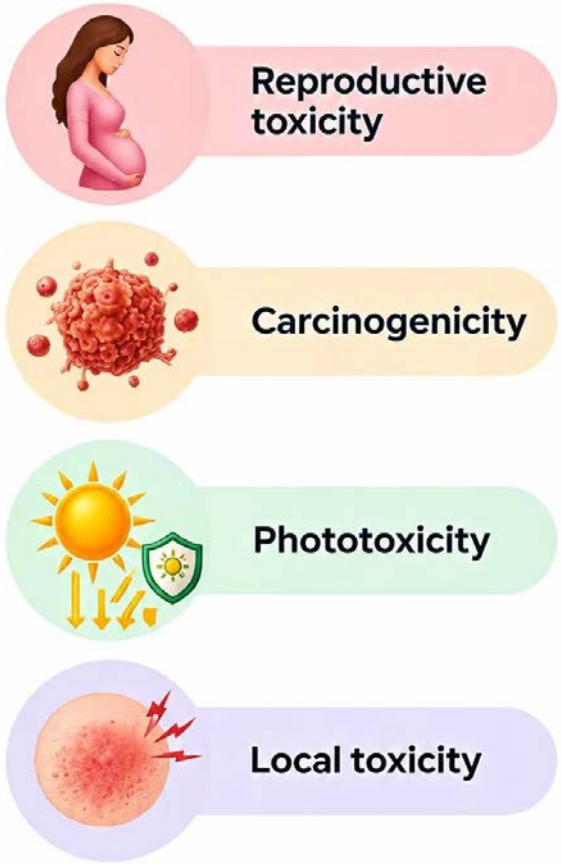
- **Cardiotoxicity (ICH S7B):**
  - Assessment of *hERG* potassium channel blockade (*in vitro*) and ECG profiling (*in vivo*) to prevent fatal QT prolongation and arrhythmias.
- **Hepatotoxicity (DILI - Drug-Induced Liver Injury):**
  - Continuous monitoring of liver enzymes (ALT, AST, AlkPhos, Bilirubin) coupled with histopathological scoring for steatosis or necrosis.
- **Nephrotoxicity:**
  - Serum biomarker tracking (e.g. Creatinine) and urinalysis to detect proximal tubule damage.
- **Immunotoxicity (ICH S8):**
  - Assessment of lymphoid organ weights (spleen, thymus), absolute lymphocyte counts, and antibody response assays.
- **Neurotoxicity (ICH S7A):**
  - Functional Observational Battery (FOB) to monitor behavioral, coordination, and motor activity changes in animals.



Specific Toxicity: Reproductive, Carcinogenic & Local

Evaluating Long-Term and Specialized Risks

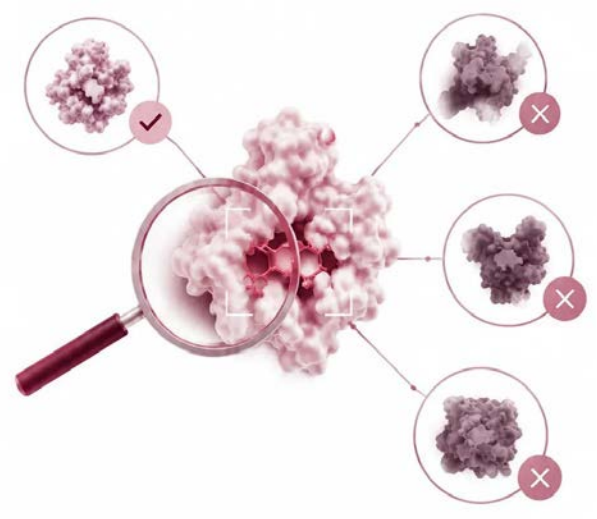
- **Reproductive Toxicity (ICH S5):**
  - *Fertility:* Effects on gametes and conception performance (rats).
  - *Teratogenicity:* Structural embryo-fetal defects evaluated in **two species** (rats and rabbits).
  - *Postnatal Development:* Late-stage pregnancy, lactation, and F1 generation reproductive fitness.
- **Carcinogenicity (ICH S1A-C):**
  - Mandatory for drugs intended for clinical use exceeding **6 months**. Requires 2-year (lifetime) bioassays in two rodent species.
- **Phototoxicity (ICH S10):**
  - Evaluation of light-induced tissue damage for molecules absorbing light in the 290–700 nm UV/visible spectrum.
- **Local Tolerance:**
  - Evaluation of tissue reactions at the exact site of administration (e.g., injection site phlebitis, dermal irritation, or ocular tolerance).



Off-Target Profiling & Anti-Targets

Safety Panels and Molecular Risk Prevention

- **Concept of Anti-Targets:**
  - Human proteins, receptors, or channels where unintended drug binding triggers severe side effects or clinical failures.
- **Safety Profiling Panels:**
  - Standardized *in vitro* radioligand assays (e.g., Eurofins/Cerep 44 or 87 panels) evaluating cross-reactivity with CNS receptors and vital enzymes.
- **Critical Hazards:**
  - *hERG Channel:* The most critical anti-target for cardiac safety (**ICH S7B**).
  - *BSEP Transporter:* Bile Salt Export Pump inhibition, highly predictive of clinical liver injury.
  - *5-HT2B Receptor:* Agonism strongly linked to drug-induced cardiac valvular fibrosis.
- **In Silico Off-Target Prediction:**
  - AI-driven models, docking and pharmacophore screening to filter out hERG blockers and cross-reactive compounds before chemical synthesis.



Public Platforms for Fast *In Silico* Screenings

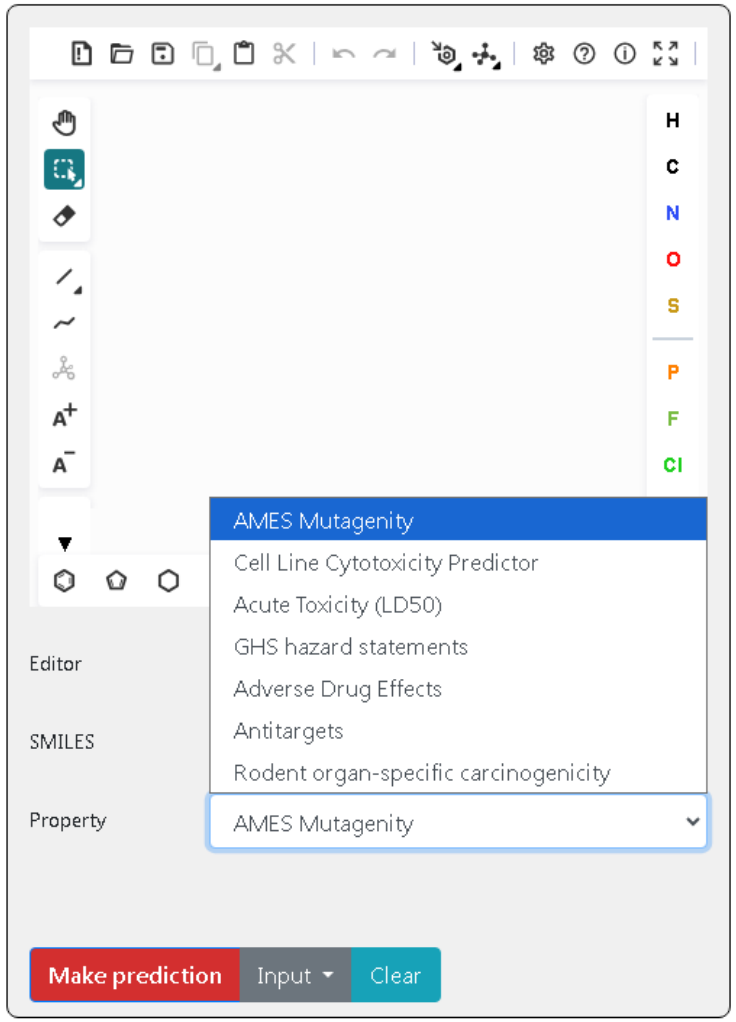
- **SwissADME / SwissTargetPrediction (SIB):**
  - Academic web server by the Swiss Institute of Bioinformatics for quick physicochemical profiling and off-target prediction.
- **proTox-3 (Charité Berlin):**
  - ML-powered platform predicting acute toxicity classes, organ-specific toxicity (hepatotoxicity, nephrotoxicity), and toxicological pathways.
- **admetSAR 2.0:**
  - Public online interface featuring optimized models for predicting hERG channel blocking and cytochrome P450 inhibition profiles.
- **OECD QSAR Toolbox:**
  - Official regulatory software developed by the OECD and ECHA for chemical grouping and read-across assessments accepted by global authorities.

Commercial Platforms

- **Lhasa Nexus (Derek & Sarah):**
  - Commercial software used by global pharma to generate regulated, expert-system compliance reports for **ICH M7**.
- **Simulations Plus:**
  - Advanced AI/ML software (ADMET Predictor®) for high-throughput screening of hERG, liver (DILI), and kidney injury.

PRIS: Preclinical Researches *in silico*

Home | Training Sets | Products/Services | Interpretation | Contacts



PRIS is a web-application for preclinical researches in silico.

This web application is prototype of future PRIS service.

The full version of curent possibility see here:

Ames test outcomes for given structural formulas across a comprehensive panel of bacterial strains.

CLC-Pred prediction of the cytotoxicity of a compound

GHS-Pred prediction of GHS Hazard Statement and Pictogram Labels of a compounds

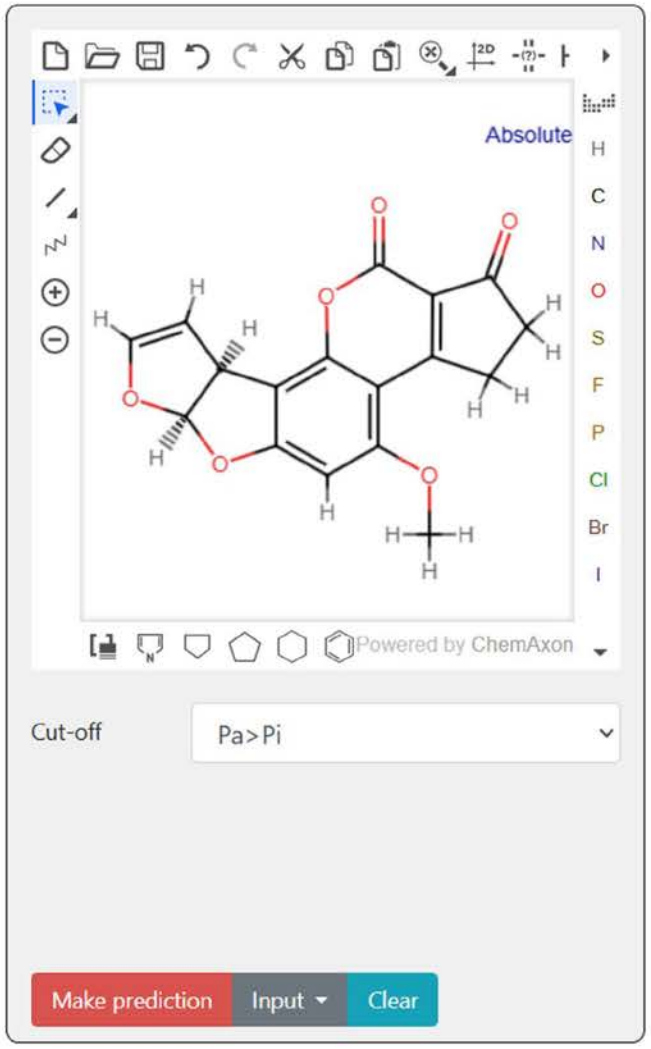
ADVERPred prediction of serious adverse drug effects of a compounds

ROSC prediction of rodent organ-specific carcinogenicity of a compounds

- **SAR models** are based on PASS technology
- **QSAR models** are based on GUSAR and SARMath software

https://way2drug.com/PRIS/

Ames Mutagenicity Predictor (prediction results for Aflatoxin B1)



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Pa	Pi	Bacterial Strains
0.868	0.003	Mutagenic (unspecified)
0.827	0.012	TA100
0.596	0.017	TA98
0.525	0.037	TA1538

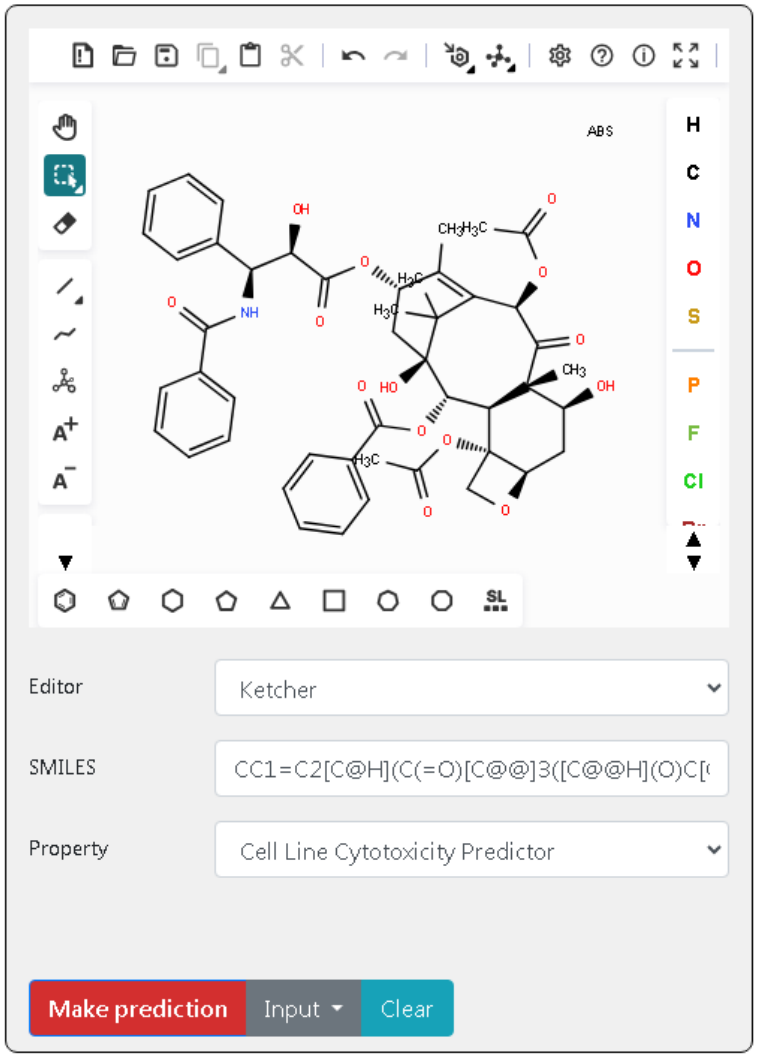
- 69 strain-specific of *Salmonella typhimurium SAR* models with an average AUC (LOO CV) - 0.944;
- Unspecified mutagenicity prediction, AUC (LOO CV) - 0.962

Pa Pi Search Bacterial Strains

Showing 1 to 4 of 4 entries Previous 1 Next

Pharmaceuticals **2025**, *18*(12), 1853; https://doi.org/10.3390/ph18121853

Prediction of pIC_{50}, pGI_{50} values for non-tumor and tumor cell lines used in toxicology (paclitaxel)



Normal cells (pIC50, pGI50) | Tumor cells (pIC50, pGI50)

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Cell line	Source	Type	Value	AD
A-431	Epithelium	pGI50	8.6280	not in AD
A-431	Epithelium	pIC50	8.1192	in AD
Caco-2	Colon	pIC50	7.3800	in AD
HCT-8	Colon	pIC50	7.2924	not in AD
HepG2	Liver	pIC50	7.1725	not in AD
THP-1	Blood	pGI50	5.3845	in AD
THP-1	Blood	pIC50	5.6307	in AD
U-937	Lymphoid	pGI50	8.3952	in AD
Cell line	**Source**	**Type**	**Value**	**AD**

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Int. J. Mol. Sci. **2025**, *26*(24), 12063; https://doi.org/10.3390/ijms262412063

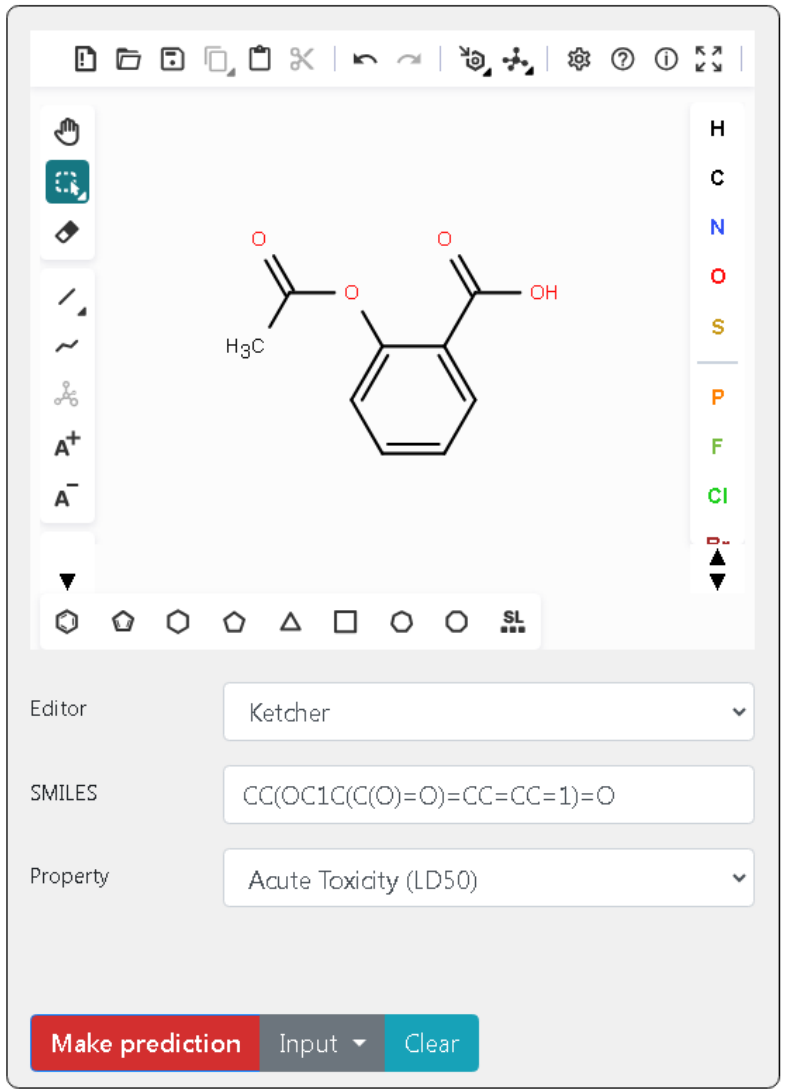
Prediction of pIC_{50}, pGI_{50} values for non-tumor and tumor cell lines used in toxicology (paclitaxel)

Cell Lines	Tissue	Endpoint	Paclitaxel	Trametinib	Dabrafenib
		Non-tumor cell lines			
GES1	Stomach	pIC_{50}	7.825	4.979	4.848
HaCaT	Skin	pIC_{50}	7.459	5.187	4.924
HEK293	Kidney	pIC_{50}	6.699	5.329	5.269
HEK-293T	Kidney	pIC_{50}	7.716	5.914	5.130
HUVEC	Endothelium	pGI_{50}	8.436	5.250	5.266
HUVEC	Endothelium	pIC_{50}	7.141	6.743	5.893
MCF-10A	Mammary gland	pIC_{50}	3.803	5.566	5.111
MRC5	Lung	pIC_{50}	9.063	4.953	5.030
PBMC	Blood	pIC_{50}	5.489	6.502	7.128
TERT-RPE1	Retina	pIC_{50}	5.117	5.564	5.293
WI-38	Lung	pIC_{50}	7.036	5.652	4.721
Mean value			6.889	5.604	5.328

Cell Lines	Tissue	Endpoint	Paclitaxel	Trametinib	Dabrafenib
		Tumor cell lines			
A-431	Skin	pGI_{50}	8.628	6.182	5.716
A-431	Skin	pIC_{50}	8.119	5.911	5.334
Caco-2	Colon	pIC_{50}	7.380	5.258	5.186
COLO205	Colon	pIC_{50}	8.470	7.579	8.140
HCT-8	Colon	pIC_{50}	7.292	6.196	6.684
HepG2	Liver	pIC_{50}	7.173	5.394	5.509
SW-620	Colon	pIC_{50}	7.519	7.030	5.656
THP-1	Blood	pGI_{50}	5.385	5.269	5.649
THP-1	Blood	pIC_{50}	5.631	7.157	6.556
U-937	Lymphoid	pGI_{50}	8.395	5.773	6.309
U-937	Lymphoid	pIC_{50}	8.536	6.906	6.566
Mean value			7.502	6.241	6.119

Int. J. Mol. Sci. **2025**, *26*(24), 12063; https://doi.org/10.3390/ijms262412063

Prediction of LD_{50} values for rodents (aspirin)



Mol WT=180.042258736

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pLD50(M)	LD50(mg/kg)	Species	Route of administration
2.028865	1684.65	rat	oral
2.247051	1019.35	mouse	subcutaneous
2.724172	339.78	rat	intraperitoneal
2.851738	253.3	rat	intravenous
2.878506	238.16	mouse	intravenous
3.346740	81.03	rabbit	intravenous
pLD50(M)	LD50(mg/kg)	Species	Route of administration

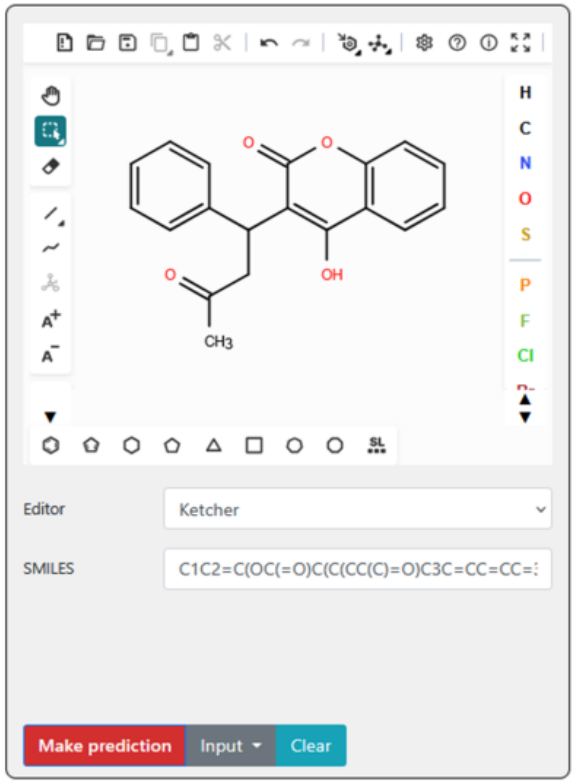
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Experimental data:

rat	LD50	oral	200 mg/kg
rat	LD50	intraperitoneal	340 mg/kg
mouse	LD50	subcutaneous	1020 mg/kg

R^2 (5-fold CV) from 0.508 (rat p.o.) to 0.695 (mouse i.v.). PubChem Data.

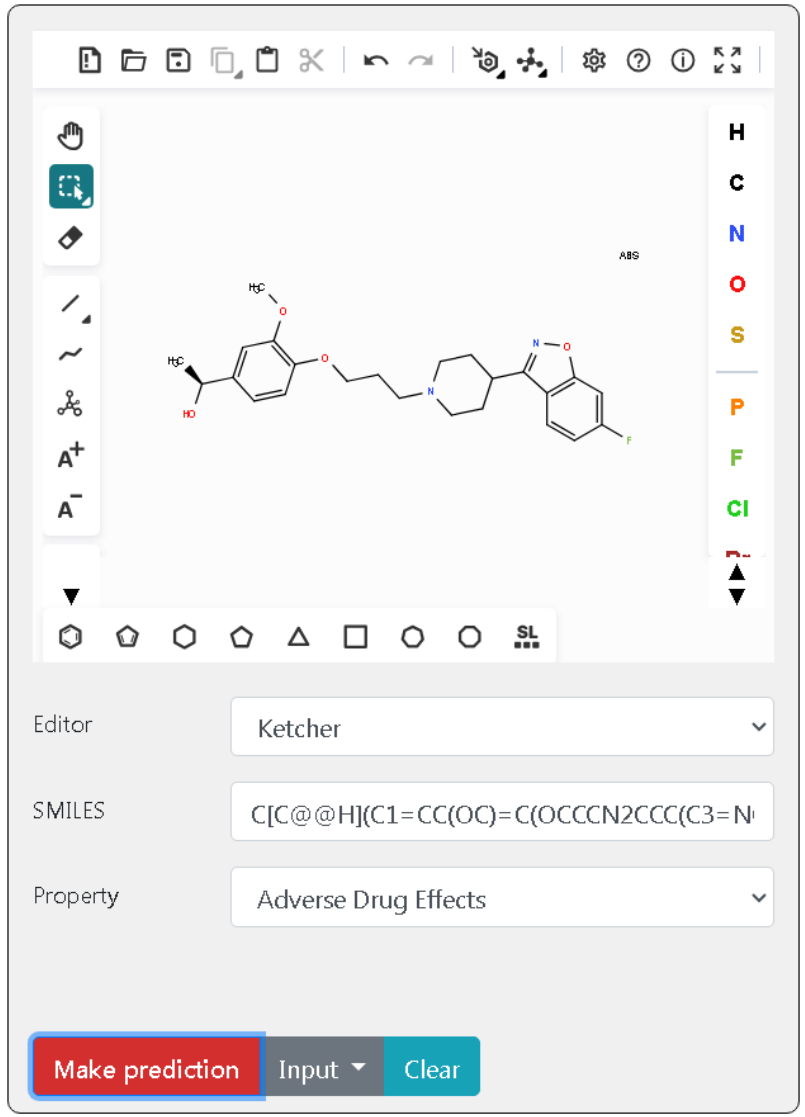
Prediction of GHS hazard statements (Warfarin)



Pa	Pi	GHS	Hazard Statements	Hazard Class
0.915	0.011	H372	Causes damage to organs through prolonged or repeated exposure	Specific target organ toxicity, repeated exposure
0.795	0.008	H360D	May damage the unborn child	Reproductive toxicity
0.775	0.037	health hazard		
0.758	0.017	H310	Fatal in contact with skin	Acute toxicity, dermal
0.749	0.017	H360	May damage fertility or the unborn child	Reproductive toxicity
0.747	0.049	H373	May causes damage to organs through prolonged or repeated exposure	Specific target organ toxicity, repeated exposure
0.673	0.045	H330	Fatal if inhaled	Acute toxicity, inhalation
0.672	0.068	H370	Causes damage to organs	Specific target organ toxicity, single exposure
0.648	0.074	acute toxicity		
0.584	0.025	H300+H310+H330	Fatal if swallowed, in contact with skin or if inhaled	Acute toxicity, oral; acute toxicity, dermal; acute toxicity, inhalation
0.480	0.111	H362	May cause harm to breast-fed children	Reproductive toxicity, effects on or via lactation
0.477	0.056	H300	Fatal if swallowed	Acute toxicity, oral
0.424	0.180	H413	May cause long lasting harmful effects to aquatic life	Hazardous to the aquatic environment, long-term hazard
0.355	0.297	H301	Toxic if swallowed	Acute toxicity, oral

It predicts 52 GHS (Globally Harmonized System) hazard statements and 4 hazard class pictograms (Acute Toxicity, Corrosives, Environmental Hazard, and Health Hazard) with Balanced Accuracy of 0.728 and 0.703 (5-fold CV), respectively. Training set was created from PubChem (~11 000 compounds).

Prediction of Adverse Drug Effects (Milsaperidone)



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Pa	Pi	Effect	Route of administration	IAP,LOO CV
0.935	0.010	QT prolongation	Oral	0.778
0.868	0.009	Stroke	Injection	0.892
0.785	0.013	Stroke	Oral	0.780
0.749	0.019	Leukopenia	Oral	0.803
0.539	0.110	Suicide	Oral	0.804
0.468	0.111	Ventricular tachycardia	Oral	0.804
0.410	0.122	Leukopenia	Injection	0.840
0.399	0.216	Depression	Oral	0.781

Prediction for 14 most serious ADRs and 2 routes of administration (including cardiovascular, hematological, psychiatric, and skin reactions, as well as DRESS syndrome, hepatotoxicity, and nephrotoxicity) with average AUC (5-fold CV) of 0.780 and a balanced accuracy of 0.714. Manually curated datasets.

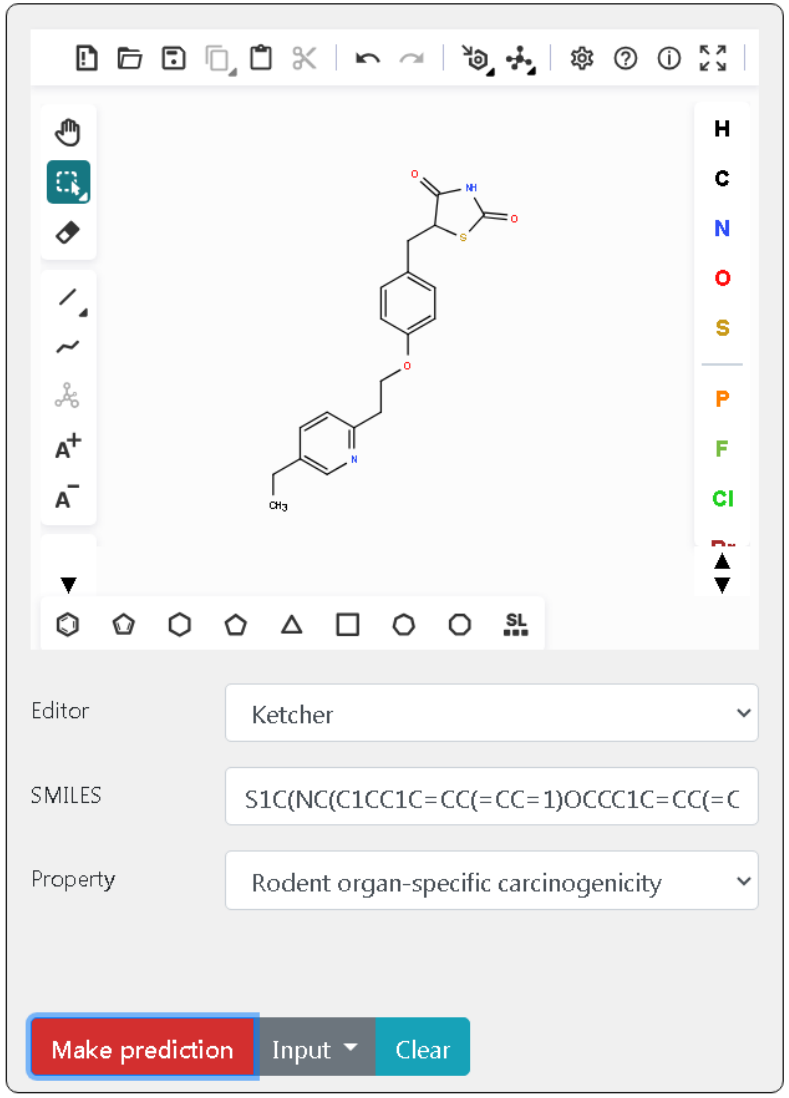
Prediction of Antitargets (Milsaperidone)

Pa	Pi	Effect	Event
0.898	0.003	Dopamine D2 antagonist	Catalepsy (acute); Convulsions (acute); Decreased Locomotor Activity (acute); Drowsiness (general); Drowsiness (acute); Increased Blood Pressure (acute); Increased Gastrointestinal Motility (general); Increased Gastrointestinal Transit (acute); Increased Urinary Sodium Excretion (acute); Increased Urine Excretion (acute); Orthostatic Hypotension (general); Orthostatic Hypotension (acute)
0.848	0.003	5 Hydroxytryptamine 2B antagonist	Cardiac Development Effect Leading To Cardiomyopathy (Based On Knockout Mouse Data) (developmental toxicity); Cardiovascular Malformations (developmental toxicity); Decreased Gastrointestinal Transit (acute); Decreased Pain (acute); Possible Cardiac Effects, Especially During Embryonic Development (developmental toxicity)
0.823	0.003	5 Hydroxytryptamine 2C antagonist	Decreased Anxiety (acute); Decreased Convulsions (acute); Drug Abuse/Dependence (acute); Increased Memory (acute)
0.775	0.003	5 Hydroxytryptamine 2A antagonist	Decreased Body Temperature (acute); Decreased Inflammation (acute); Decreased Pain (acute); Decreased Platelet Aggregation (acute); Increased Sleep (acute)
0.604	0.007	HERG channel blocker	QT Prolongation (general); Reduced, Survival
0.535	0.004	5 Hydroxytryptamine 1A antagonist	Anxiety (general); Decreased Anxiety (acute); Decreased Sleep (acute); Dizziness (acute); Increased Locomotor Activity (acute); Increased Memory (acute); Paresthesia (acute); Visual Disturbances (acute)
0.510	0.003	Dopamine D1 antagonist	Anti-Emetic Effect (general); Anxiety (general); Anxiety (acute); Catalepsy (acute); Decreased Convulsions (acute); Decreased Locomotor Activity (acute); Depression (general); Depression (acute); Dyskinesia (general); Dyskinesia (acute); Increased Blood Pressure (acute); Parkinsonism (general); Parkinsonism (acute); Suicidal Intent (general); Suicidal Intent (acute)
0.492	0.004	Alpha 1a adrenoreceptor antagonist	Blood Pressure Events (general); Chronic Heart Failure (acute); Decreased Blood Pressure (acute); Decreased Smooth Muscle Tone (general); Dizziness (general); Dizziness (acute); Increased Heart Rate (general); Increased Heart Rate (acute); Orthostatic Hypotension (general); Orthostatic Hypotension (acute); Orthostatic Hypotension (general); Retrograde Ejaculation (acute); Sexual Dysfunction (general)
0.454	0.004	Dopamine D3 antagonist	Insomnia; Nausea; Severe Akathisia
0.411	0.004	Alpha 1b adrenoreceptor antagonist	Decreased Blood Pressure (acute)
0.407	0.087	Calcium channel L-type activator	Convulsions (acute); Decreased Locomotor Activity (acute); Increased Blood Pressure (acute); Increased Cardiac Contractility (acute); Increased Mortality In Heart Failure Patients (general); Increased Systolic Blood Pressure (general); Increases In Peripheral Vascular Resistance (general); Seizures (general)

Many predicted MOAs correspond to known targets of Milsaperidone including dopamine, serotonin, and adrenergic receptors which influence cardiovascular and central nervous systems (see “event” column).

Prediction of 312 safety pharmacology-related molecular mechanisms of action (MOAs) with established links to off-target toxicities with average AUC (5-fold CV) - 0.976

Rodent organ-specific carcinogenicity (pioglitazone)



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Pa	Pi	Organs	Species	Sex
0.472	0.259	thyroid gland	female	mice
0.442	0.257	urinary bladder	male	mice
0.439	0.222	thyroid gland	male	mice
0.426	0.238	kidney	male	mice
0.378	0.244	liver	male	mice

- Prediction of rodent carcinogens considering the species (rats, mice), sex, and tissue-specificity;
- The data from Carcinogenic Potency Database for 1011 organic compounds evaluated on the standard two-year rodent carcinogenicity bioassay;
- The average prediction accuracy (AUC) for training sets is 79% (LOO CV) and 78.2% (10-fold CV).

Bioinformatics. **2018**, 34(4):710-712. https://doi.org/10.1093/bioinformatics/btx678

Acknowledgments

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