

WEBINAR

Métodos Alternativos ao uso
de animais para indústria de
Higiene Pessoal, Perfumaria e
Cosméticos

Modelos *in silico* e integração de AOPs na avaliação de ingredientes cosméticos com fins regulatórios

São Paulo
2023

Overview

- **Sobre nós e nossos trabalhos**
- **Entendendo os modelos *in silico* e alguns inteligência artificial**
- **Contexto regulatório: guias e exemplos de recomendação legal de modelos *in silico***
- **Exemplos de aplicação: AOPs / NGRA / IATA / Defined Approaches**

Sobre a Alttox

Fundada em **2012** e localizada na Av. Vital Brasil, 305 – Butantã, São Paulo – SP.



Responsável pela **primeira plataforma brasileira de modelos *in silico*** validados com fins regulatórios do Brasil e América Latina, em projeto financiado pela FAPESP, a Is-Tox Platform.



- Mais de **70 clientes** atendidos e inúmeros projetos de sucesso
- Pioneira nos modelos *in silico* no Brasil com fins regulatórios

Parceiro estratégico e renomado para softwares e avaliações *in silico* de segurança



Premiações de inovação e pesquisa em congressos e associações acadêmicas.



Softwares e projetos de pesquisa e inovação

- 1ª plataforma de modelos QSAR *in silico* do Brasil e América Latina → Real time



Contaminantes

Soluções em avaliação toxicológica de impurezas e contaminantes



Cosméticos

Estudos sem o uso de animais e com base na estrutura química



Novas Moléculas

Soluções em avaliação toxicológica de novas moléculas e ingredientes



Medicamentos

Soluções desenhadas para atender às legislações específicas



Agroquímicos

Relatórios de avaliação de toxicidade impurezas para IBAMA, MAPA e ANVISA



Alimentos

Avaliação toxicológica dos potenciais contaminantes neoformados (NFCs)



IrriTest™



Pred-Oral™



Genotox-iS™



AOP-Sens™



iS-Liver™



Pred-CYP22D™



iS-Ocular™



Acute-Tox™



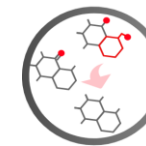
DevTox-iS™



BCF-Test™



Pred-Ecotox™



iS-Purge



DegradationPlot
Prediction of degradation products and pathways



NitroRisk
Risk Assessment for N-nitrosamines formation



Prioritizer
Ranking of priority for nitrosamines risk assessment of drug substances



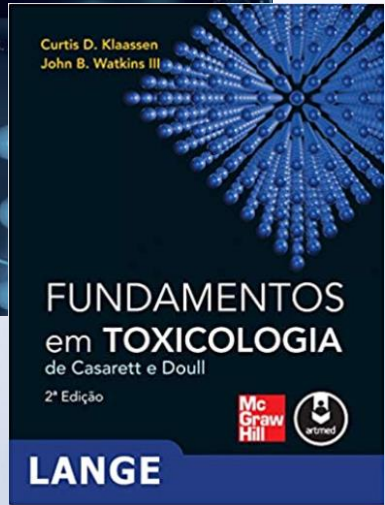
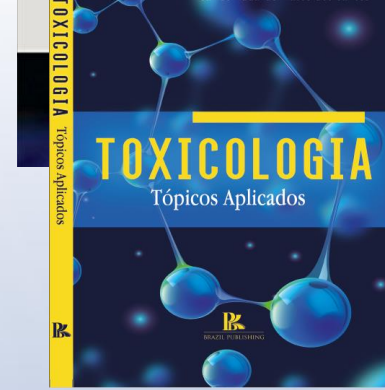
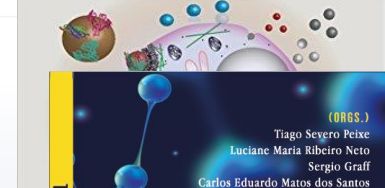
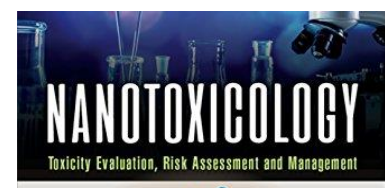
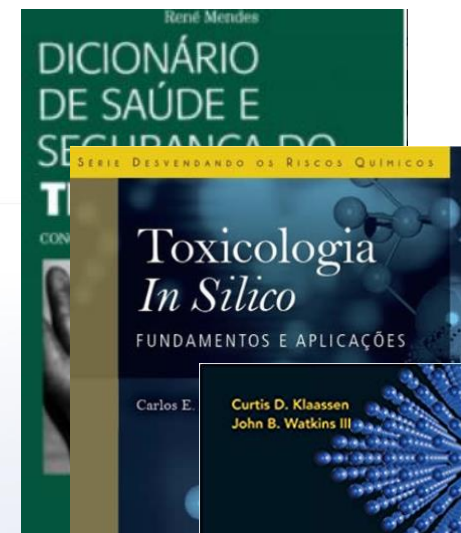
Projeto financiado:



<https://is-tox.com/our-models>



ALGUMAS PUBLICAÇÕES



Regulatory Toxicology and Pharmacology 136 (2022) 105288

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

Journal homepage: www.elsevier.com/locate/yrtph

Pharmaceutical Medicine
<https://doi.org/10.1007/s40290-020-00357-6>

LETTER TO THE EDITOR

Setting limits for N-nitrosodimethylamine (NDMA) in read-across and structural analogues of N-nitrosopiperazine

Carlos E. Matos dos Santos^{a,*}

^a Altos Ltda, São Paulo, SP, Brazil
^b Department of Chemistry, Faculty of Philosophy, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Handling Editor: Dr. Martin Van den berg

Keywords:
Nitrosamines
Mutagenic inquiry
Risk assessment approach
Read-across
in silico

Dear Editor,

We read the review by Valeur et al. [1] about the safety of excipients in neonatal medicinal products, with many interesting points and an objective discussion about access to knowledge on the clinical pharmacology of excipients in neonates and its impact in improving the quality of risk assessment and decision making during drug development by a risk-benefit analysis. Although we think the article discusses relevant aspects about the safe use of excipients in medicines used to treat newborns, we have a few concerns, considering the importance of adequate interpretation of toxicological limits for the quality of risk assessments, and regulatory and/or clinical decisions.

First, in Table 2, the authors provided an overview of the supposed tolerance limits for ethanol, propylene glycol and benzyl alcohol as proposed by the European Medicines Agency (EMA) [2-4], highlighting a limit for benzyl alcohol of 5 mg/kg/day "for adults and children aged over 4 weeks". However, this limit is not for benzyl alcohol, but for the benzoic acid and its salts (e.g. sodium benzoate and other benzoates) orally in food [2]. Second, this is not a limit proposed or established by the EMA as was described in Table 2. This limit is only cited by the EMA, and as described in the EMA's report [2] is an Acceptable Daily Intake proposed by the JECFA (Joint FAO/WHO Expert Committee on Food Additives) [5].

Most importantly, contrary to the considerations about tolerance limits presented by the authors, no specific limits were set in the EMA's report for the excipient benzyl alcohol.

* Corresponding author. Altos Ltda, São Paulo, SP, Brazil. E-mail address: carlosmatos@usp.br

<https://doi.org/10.1016/j.yrtph.2022.105288>
Received 9 July 2022; Received in revised form 13 November 2022; Accepted 20 October 2022

Published online: 20 October 2022

MDPI

Article

Challenges and Opportunities for Integrating *In Silico* Models and Adverse Outcomes Pathways to Set and Relate New Biomarkers

Carlos E. Matos dos Santos^{1,2,*}, Raul Ghiraldelli Miranda³, Danielle Palma de Oliveira¹ and Daniel Junqueira Dorta³

¹ School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP 14040-903, Brazil; dpalma@usp.br
² Altos Ltda, Brazil—Alternative Toxicology, São Paulo, SP 05503-001, Brazil
³ Department of Chemistry, Faculty of Philosophy, Sciences and Letters of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP 14040-903, Brazil; raul.miranda@usp.br (R.G.M.); djdorta@fclrp.usp.br (D.J.D.)
* Correspondence: carlosematos@usp.br

Received: 21 October 2020; Accepted: 4 December 2020; Published: 17 December 2020

check for updates

Abstract: The Adverse Outcome Pathway (AOP) framework has been considered the most innovative tool to collect, organize, and evaluate relevant information on the toxicological effects of chemicals, facilitating the establishment of links between molecular events and adverse outcomes at the critical level of biological organization. Considering the combination of the high volume of toxicological and ecotoxicological data produced and the application of artificial intelligence algorithms from the last few years, not only can higher mechanistic interpretability be reached with new *in silico* models, but also a potential increase in predictivity in hazard assessments and the identification of new potential biomarkers can be achieved. The current paper aims to discuss some potential challenges and ways of integrating *in silico* models and AOPs to predict toxicological effects and to set and relate new biomarkers for defined purposes. With the use of the AOP framework to organize the ecotoxicological, toxicological, and structural data generated from *in chemico*, *in vitro*, *ex vivo*, *in vivo*, and population studies, it is expected that the generated biological and chemical construct will improve its application, establishing a knowledge platform to set and relate new biomarkers by key event relationships (KERs).

Keywords: Adverse Outcome Pathway; *in silico* models; biomarkers; predictive toxicology

1. Introduction

In Silico Toxicology (IST) integrates different mathematical models to predict the toxicity of chemicals based on patterns of structural and physicochemical properties related to the toxicological activity.

With increasing relevance in different applications and as a cost-effective tool with a potentially higher mechanistic interpretability in evidence-driven assessments, *in silico* models (computer-based) are considered useful for predicting the toxicity of chemicals with unknown biological activity and are recommended by regulators and/or proposed by investigators for use in various contexts. Some types of substances assessed by *in silico* models in the last few years include drug impurities and degradation products [1]; pesticide metabolites and impurities [2]; additives, neo-formed contaminants (NFCs), and contaminants in food [3–5]; cosmetic ingredients [6]; industrial chemicals and emerging chemicals (ECs) [7]; and others.

Modelos *in silico*

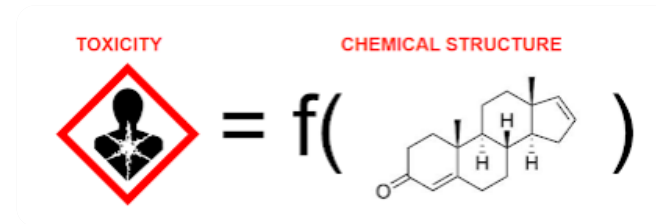
Fundamentos



O que são modelos *in silico*?

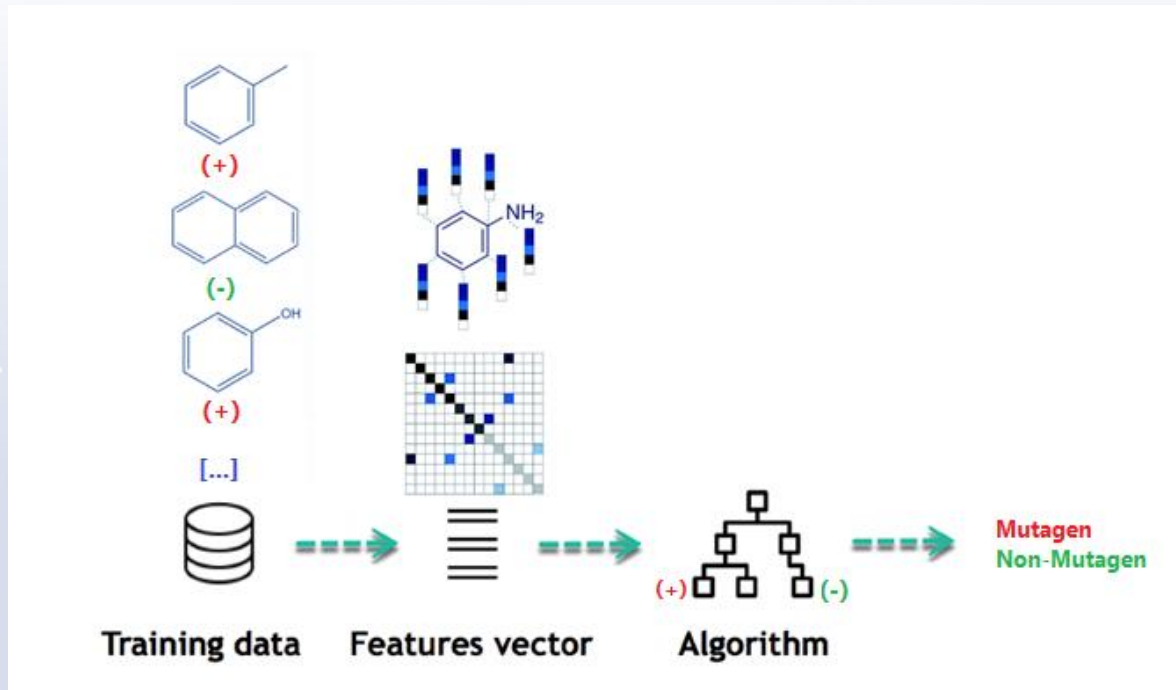
- Uma das **NAMs (New Approach Methodologies)** baseadas em uso de softwares / modelos computacionais

- SAR** – Relações Estrutura –atividade
- QSAR** – Relações Quantitativas entre Estrutura e Atividade
- (Q)SAR** – Referido-se a ambos, SAR e QSAR!
- **Métodos de extrapolação e interpolação baseada em similaridade estrutural ou mecanicista (Read-across, análise de tendência, formação de categorias e outros)**
- **Inteligência Artificial e algoritmos de machine learning (RF, redes neurais, kNN, SVM, Deep Learning etc.)**



Como prever o potencial de toxicidade?

- Dados prévios -> Identificação de padrões (relação estrutura-atividade)
- Criação de algoritmos -> Predições



Critérios de validação da OECD

- 1- Um endpoint definido
- 2- Um algoritmo inequívoco
- 3- Um domínio de aplicabilidade definido
- 4- Medidas apropriadas de qualidade de ajustamento, robustez e preditividade
- 5- Uma interpretação mecanística, se possível

Unclassified
ENV/JM/MONO(2007)2

Organisation de Coopération et de Développement Economiques
Organisation for Economic Co-operation and Development
30-Mar-2007

English, French

**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

Cancels & replaces the same document of 15 February 2007

**GUIDANCE DOCUMENT ON THE VALIDATION OF (QUANTITATIVE)STRUCTURE-ACTIVITY
RELATIONSHIPS [(Q)SAR] MODELS**

JT03224782

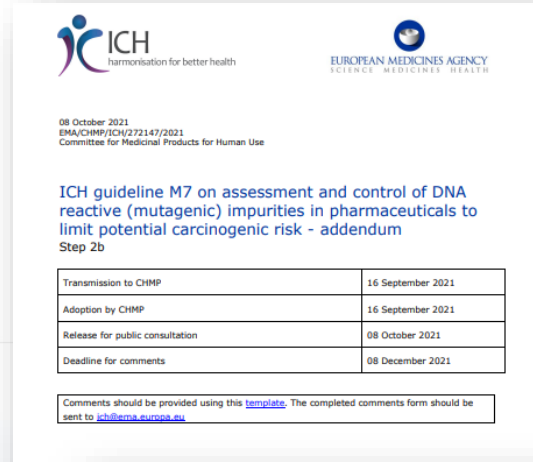
Document complet disponible sur OLIS dans son format d'origine
Complete document available on OLIS in its original format

ENV/JM/MONO(2007)2
Unclassified

English, French

Modelos *in silico* são aceitos com fins regulatórios?

- ▶ Não apenas aceitos, mas em alguns casos **exigidos**
 - ▶ **Isoladamente; ou,**
 - ▶ **Combinados com testes *in chemico*, *in vitro* e/ou *ex vivo* (New Approach Methodologies)**
- ▶ Exemplos (lista não exaustiva)
- ▶ **Guideline OECD No. 497 (2021)** Guideline on Defined Approaches for Skin Sensitisation (contextos diversos, sobretudo o cosmético)
 - ▶ Recomendável inclusão de mais métodos e modelos *in silico* validados ao invés de “softwares” (boa prática regulatória)
- ▶ **ICH M7** (contexto farmacêutico)
- ▶ **ISO 10993-23:2021** (avaliação de componentes de dispositivos médicos)
- ▶ **SCCS Notes** - Guidance for Safety Evaluation of Cosmetic Ingredients (12^a rev 2023)
- ▶ **Legislações relacionadas a inventários e registros de substâncias: REACH, TSCA, DSL**
- ▶ **E outras nos setores agroquímicos, alimentos e medicamentos;**



ICH harmonisation for better health
EUROPEAN MEDICINES AGENCY
SCIENCE · MEDICINES · HEALTH

08 October 2021
EMA/CHMP/ICH/272147/2021
Committee for Medicinal Products for Human Use

ICH guideline M7 on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk - addendum Step 2b

Transmission to CHMP	16 September 2021
Adoption by CHMP	16 September 2021
Release for public consultation	08 October 2021
Deadline for comments	08 December 2021

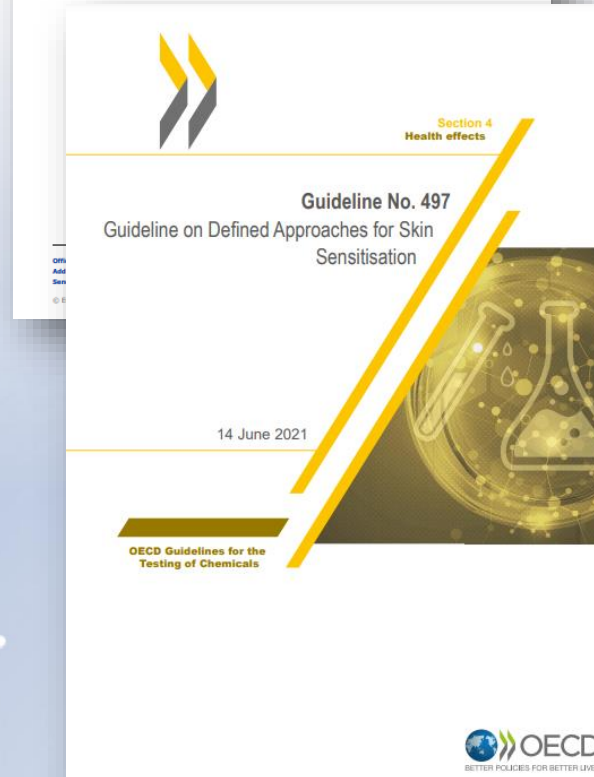
Comments should be provided using this [template](#). The completed comments form should be sent to ich@ema.europa.eu



BS EN ISO 10993-23:2021

BSI Standards Publication

Biological evaluation of medical devices Tests for irritation



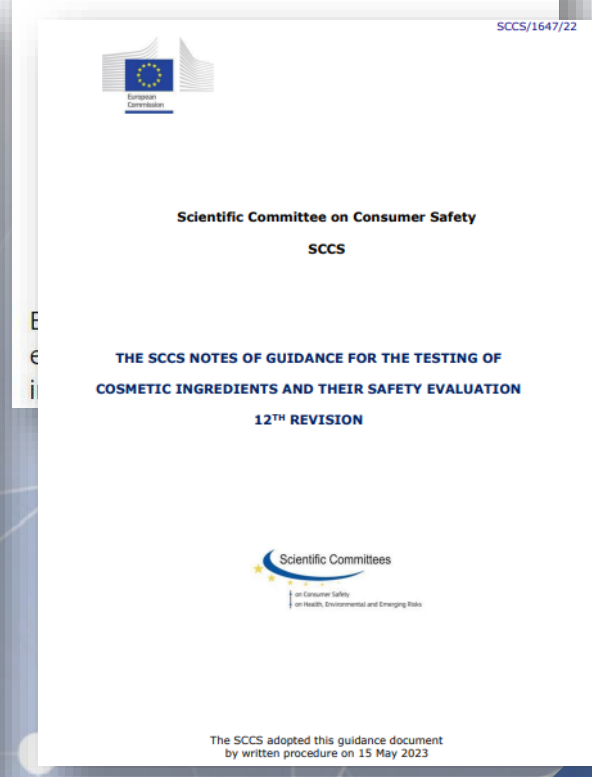
Section 4
Health effects

Guideline No. 497
Guideline on Defined Approaches for Skin Sensitisation

14 June 2021

OECD Guidelines for the Testing of Chemicals

OECD
BETTER POLICIES FOR BETTER LIVES



SCCS/1647/22

European Commission

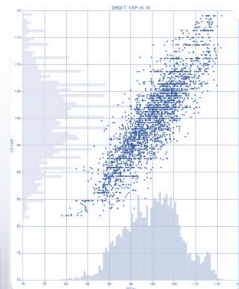
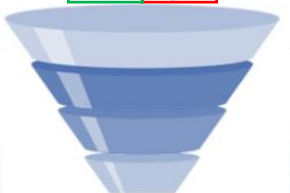
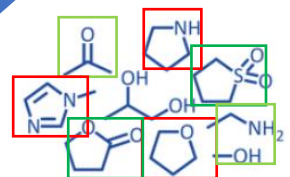
Scientific Committee on Consumer Safety
SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION
12TH REVISION

Scientific Committees
on Consumer Safety
on Health, Environmental and Emerging Risks

The SCCS adopted this guidance document by written procedure on 15 May 2023

Exemplo de matriz SAR de alertas



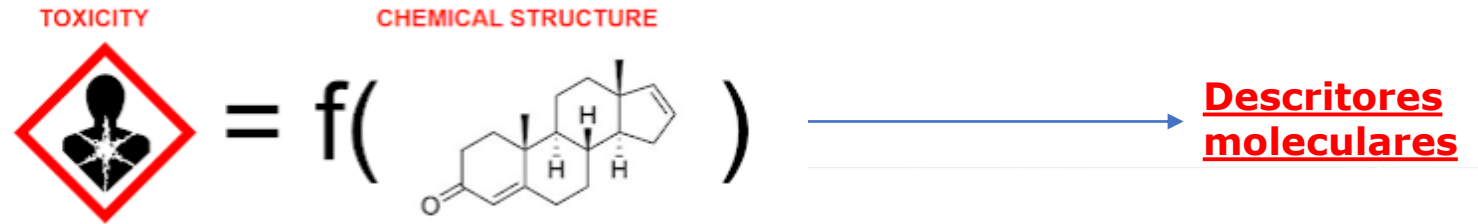
	EPs	Support	ρ	Structural Alert in ToxAlerts
MF_73		44	36.94	N-nitroso-N-alkylamides N-nitroso-N-alkylureas N-nitroso-N-alkylcarbamates
MF_0		67	27.92	Diazo
MF_1		55	22.76	Azide
MF_125		52	21.47	Aromatic and aliphatic aziridinyl derivatives
MF_156		47	12.60	Aromatic hydroxylamine ester
MF_72		27	9.09	Nitrosamine
MF_1651		178	6.79	Polycyclic aromatic hydrocarbons
MF_1841		26	6.59	Allylic halides
MF_824		48	6.01	Hydroxyl amine
MF_1667		226	5.84	Polycyclic aromatic hydrocarbons
MF_1831		36	5.33	Monohaloalkene
MF_75		162	5.19	Unsubstituted heteroatom-bonded heteroatom
MF_927		35	5.16	Aromatic N-acyl amine
MF_1298		27	4.94	Quinones
MF_920		964	4.70	Nitrosoarenes
MF_134		926	4.63	Aromatic nitro groups
MF_444		93	4.47	Nitrogen mustard

	EPs	Support	ρ	Structural Alert in ToxAlerts
MF_212		35	4.15	N mustard
MF_2188		29	4.12	Acyl halides
MF_2107		40	4.05	Alkyl ester of sulfonic and sulfuric acids
MF_34		74	3.68	Aliphatic azo
MF_1317		26	3.61	Heterocyclic polycyclic aromatic hydrocarbons
MF_1883		308	2.33	Aliphatic and aromatic epoxides
MF_916		656	2.17	Primary aromatic amine
MF_432		50	1.83	Alkyl carbamate
MF_41		151	1.54	Aromatic azo
MF_153		51	1.33	Aliphatic nitroso
MF_169		182	1.25	Tertiary aromatic amine
MF_2197		26	1.17	Aliphatic halogens
MF_1836		59	1.17	α,β-unsaturated carbonyl
MF_1470		43	0.82	Coumarins
MF_2220		1491	0.64	Simple aldehyde
MF_2097		47	0.58	Alkyl ester of phosphonic and phosphoric acids

Modelos estadísticos - algoritmos



Modelos estatísticos (QSAR)

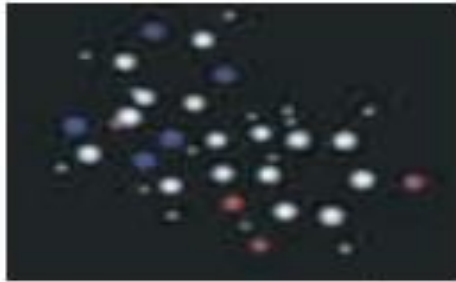


Toxicidade = f (carat. FQ e/ou estruturais)
 Quantitative structure-toxicity relationship (QSTR)

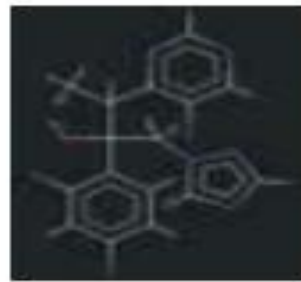
- ▶ Resposta contínua: IC50, EC50, DL50, CL50 etc;
- ▶ Respostas categóricas: +/-
- ▶ Classificatória (fraco, moderado, forte, extremamente forte etc.)
- ▶ Probabilística: 0 a 1 (0 – 100%)

Obs: a notação (Q)SAR ao invés de QSAR, é frequentemente utilizada na literatura para descrever modelos de todas as categorias, incluindo aqueles sem relação quantitativa entre estrutura e atividade, como SAR e regras.

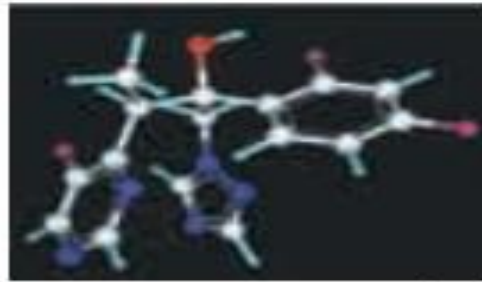
Descritores e formas de representação estrutural QSAR 1-D, 2-D, 3-D e 4-D



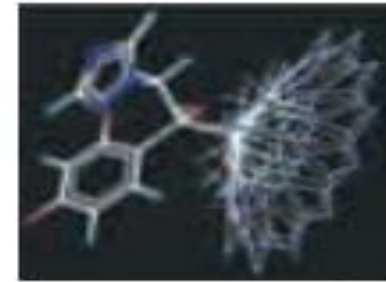
1D Representation



2D Representation



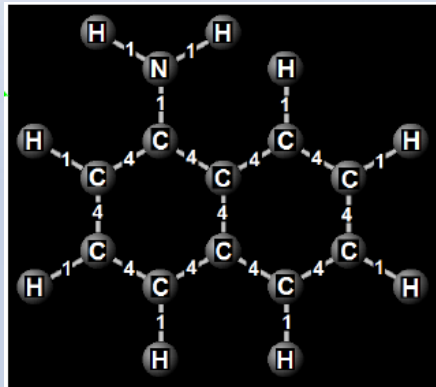
3D Representation



4D Representation

SMILES (Simplified
molecular-input line-entry system)

C1(=CC=CC2=C1C=CC=C2)N



MOL File

```

16 15 0 0 0 0 0 0 0 0999 V2000
-0.1958 -2.9667 0.0000 C 0 0 0 0
0.5147 -2.5500 0.0000 C 0 0 0 0
0.5135 -1.7250 0.0000 N 0 0 0 0
1.2292 -2.9625 0.0000 H 0 0 0 0
1.9417 -2.5458 0.0000 C 0 0 0 0
2.6542 -2.9583 0.0000 C 0 0 0 0
3.3667 -2.5417 0.0000 C 0 0 0 0
4.0792 -2.9542 0.0000 C 0 0 0 0
4.7917 -2.5375 0.0000 C 0 0 0 0
5.5042 -2.9500 0.0000 C 0 0 0 0
1.2250 -3.7875 0.0000 C 0 0 0 0
0.8083 -4.5000 0.0000 C 0 0 0 0
1.3917 -5.0833 0.0000 C 0 0 0 0
0.9750 -5.7958 0.0000 C 0 0 0 0
1.5583 -6.3792 0.0000 C 0 0 0 0
0.9708 -6.9625 0.0000 C 0 0 0 0
8 9 1 0 0 0 0 0 0 0 0 0 0 0 0
4 5 1 0 0 0 0 0 0 0 0 0 0 0 0
9 10 1 0 0 0 0 0 0 0 0 0 0 0 0
2 3 1 0 0 0 0 0 0 0 0 0 0 0 0
4 11 1 0 0 0 0 0 0 0 0 0 0 0 0
5 6 1 0 0 0 0 0 0 0 0 0 0 0 0
11 12 1 0 0 0 0 0 0 0 0 0 0 0 0
1 2 1 0 0 0 0 0 0 0 0 0 0 0 0
12 13 1 0 0 0 0 0 0 0 0 0 0 0 0
5 6 7 1 0 0 0 0 0 0 0 0 0 0 0
13 14 1 0 0 0 0 0 0 0 0 0 0 0 0
2 4 1 0 0 0 0 0 0 0 0 0 0 0 0
14 15 1 0 0 0 0 0 0 0 0 0 0 0 0
7 8 1 0 0 0 0 0 0 0 0 0 0 0 0
15 16 1 0 0 0 0 0 0 0 0 0 0 0 0
M END

```

Vertices
(atomic type,
coordinates etc.)

Edges
(connectivity table,
label-types of bonds)

Formas que a
máquina pode
entender!


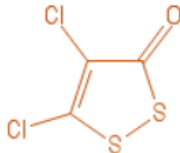


Descritores fingerprints

- Fingerprints são descritores na forma de uma lista de valores binários (0 ou 1), que podem ser gerados por diferentes Keys (MACCS Keys, ECFP etc.)
- Um conjunto de padrões estruturais podem ser checados. Por exemplo, uma estrutura **OC=CN**, pode conter:



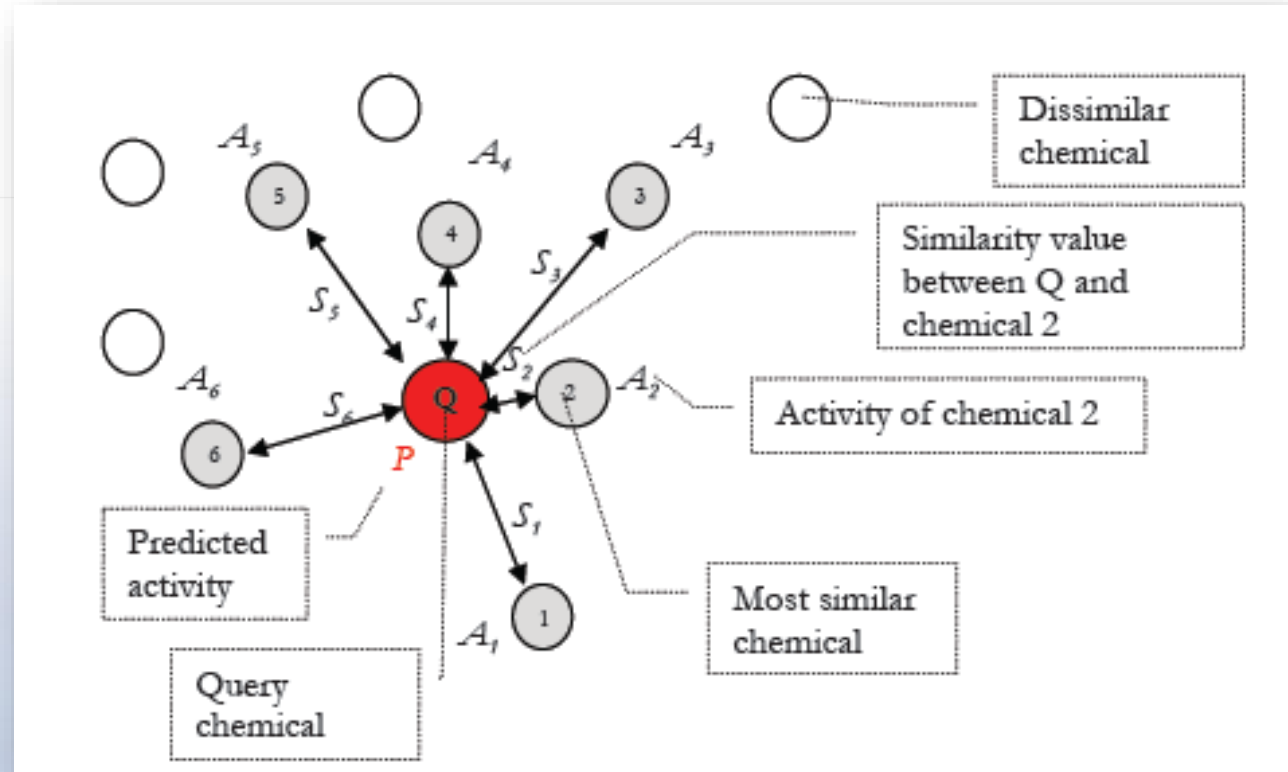
0-bond paths: **C** **O** **N**
1-bond paths: **OC** **C=C** **CN**
2-bond paths: **OC=C** **C=CN**
3-bond paths: **OC=CN**

Exemplo Fingerprint MACCS

MACCS Keys (conjunto de questões)		
Há menos de 3 oxigênios?	Yes (1)	Yes (1)
Há uma ligação S-S?	Not (0)	Yes (1)
Há um anel de 5 membros?	Yes (1)	Yes (1)
É pelo menos um F, Cl, Br, ou me apresentar?	Not (0)	Yes (1)
Lista de bits ou bitstring		

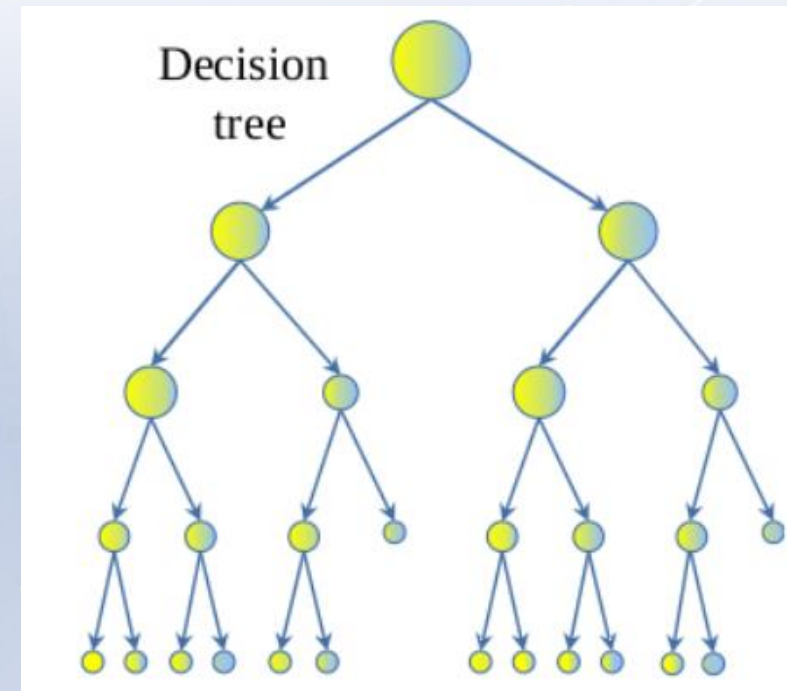
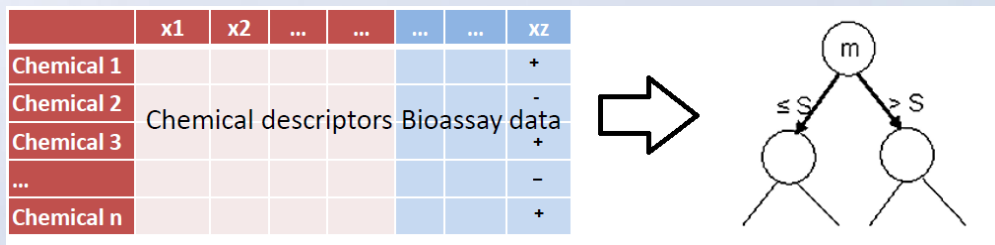
kNN (Nearest Neighbor)

- Modelo baseado em um espaço químico reduzido com **moléculas vizinhas mais próximas**
- Premissa de que moléculas similares devem ter **atividade biológica similar**
- Quando uma nova molécula é inserida, um conjunto de moléculas estruturalmente relacionadas semelhantes é obtido no banco de dados e utilizado para classificar a nova instância de consulta
- Limitações e incertezas



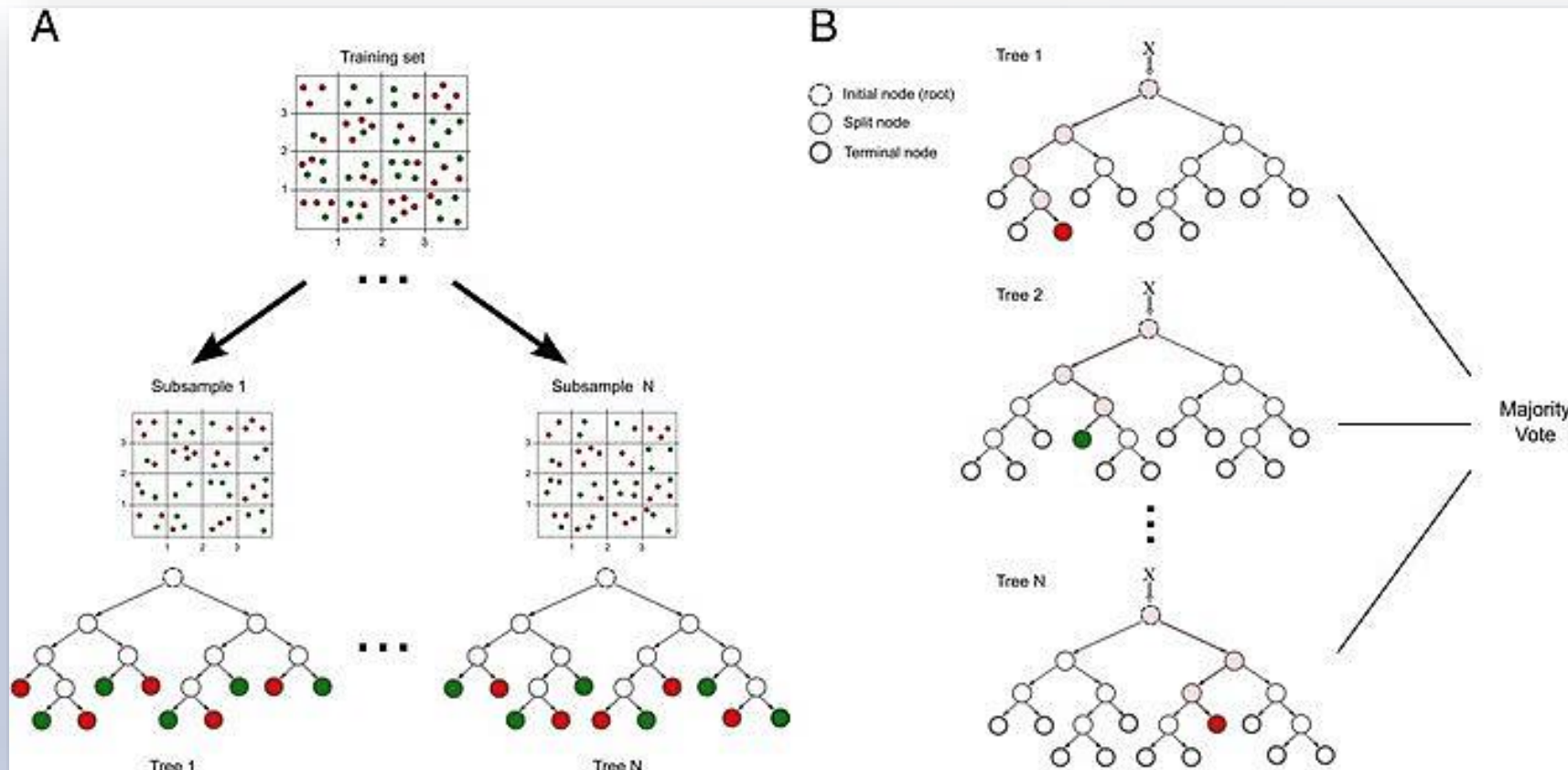
Árvores de decisão e deep decision

- CART – Classification and Regression Tree
- Procura entre todos os valores de todos os preditores a divisão em 2 grupos
 - Em classificação: garantir maior pureza em cada grupo



Random forests

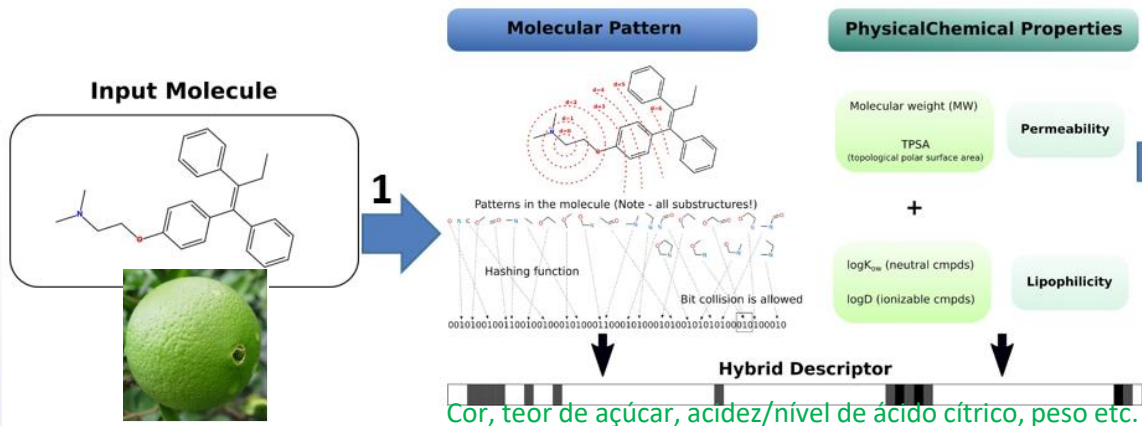
- Bagging (sorteio com aleatorização e reposição)
- Randomização na seleção de preditores em cada nó
- Menos variáveis em cada nó
- Predição segundo voto da maioria das árvores



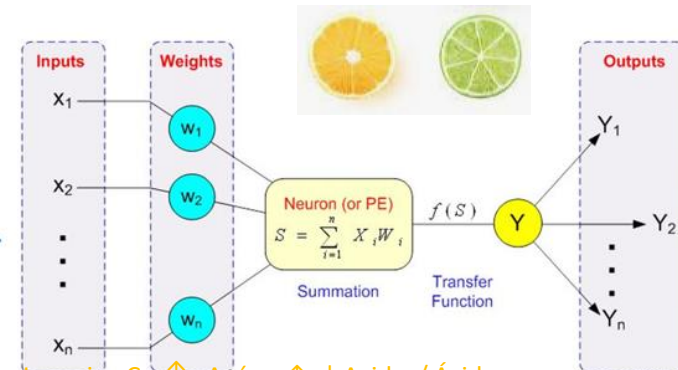
Deep Learning com laranja e limões



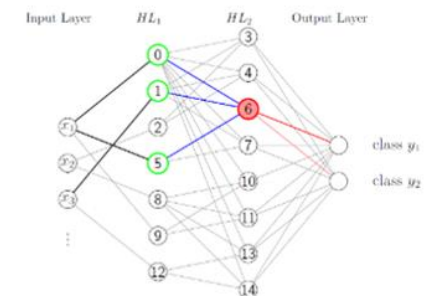
Fingerprint Híbrido
 União do fingerprint ECFP e propriedade físico-química:
 MW, TPSA, log_{Kow}, logD



deep learning (ANN)
 Machine learning decision model implemented with
 the Hybrid fingerprint

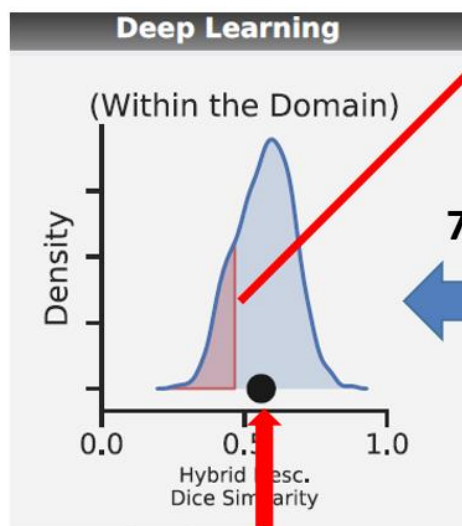


Laranja= Cor↕, Açúcar↑, ↓Acidez/ Ácido cítrico, ↕peso, caipirinha↓
 Limão= Cor↕, ↓Açúcar, ↑ Acidez/ Ácido cítrico, ↕peso, ↑ caipirinha



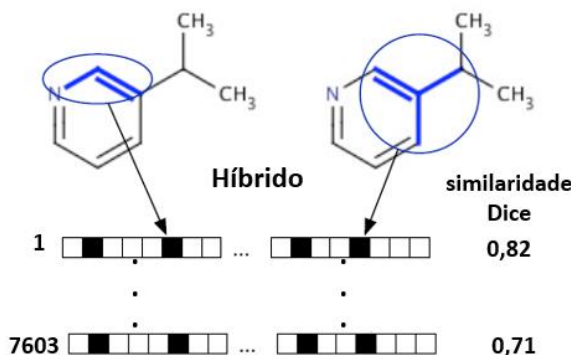
Laranja – Conf. 80%

Predição composta pelo voto majoritário. Exemplo para resultado mutagênico com 70% de confiança. Durante a amostragem externa (5-fold externo), 70% das amostras apontavam combinação de pesos nas camadas profundas de neurônio para decisão de potencial mutagênico.



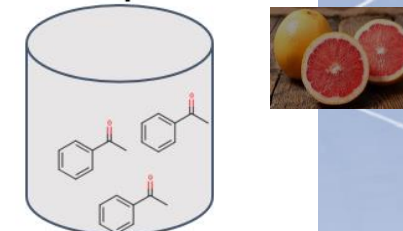
$$D_c = Z\sigma_{ec} + y_{ec}$$

Na Equação, o y_{ec} é a média aritmética e σ_{sec} o desvio padrão das similaridade dice dos k vizinhos mais próximos de cada composto na série treinamento. O parâmetro empírico Z controla o nível de significância, sendo que seu valor de 1,0. O valor de k ideal pode ser obtido pelo valor empírico calculado usando $n^{1/3}$ (n =dataset tamanho).



1. Cálculo da similaridade da matriz de dados contra ela mesma para gerar uma matriz quadrada.
2. cálculo da similaridade dice entre a "input molecule" e todas as substâncias da dataset.

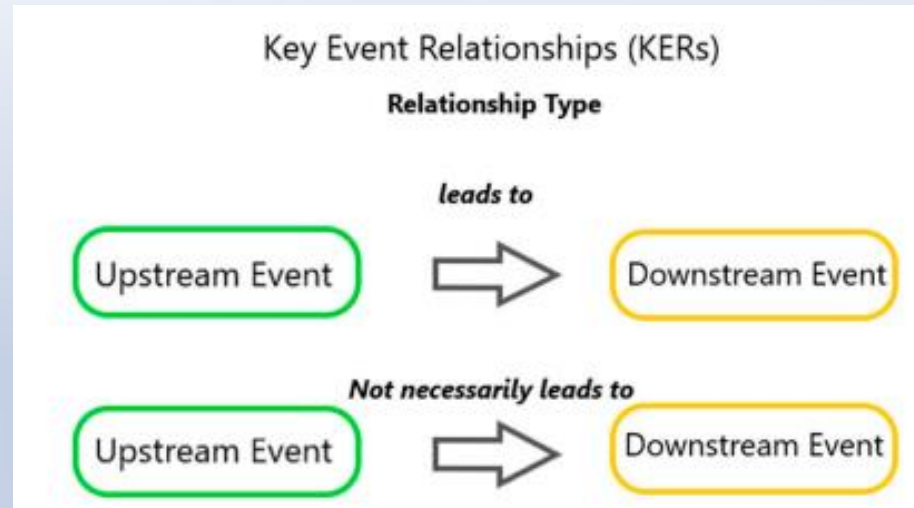
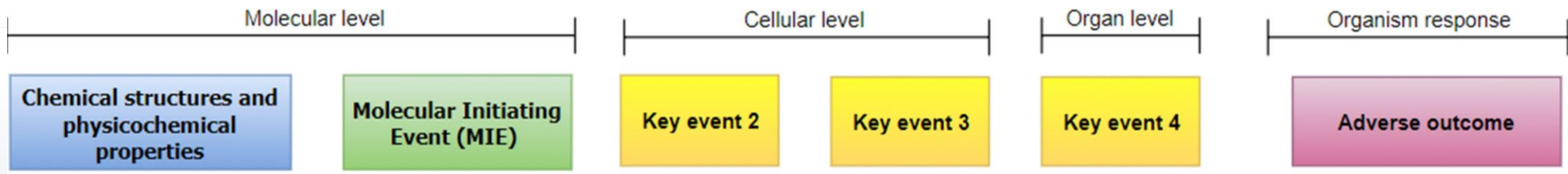
Domínio de aplicabilidade



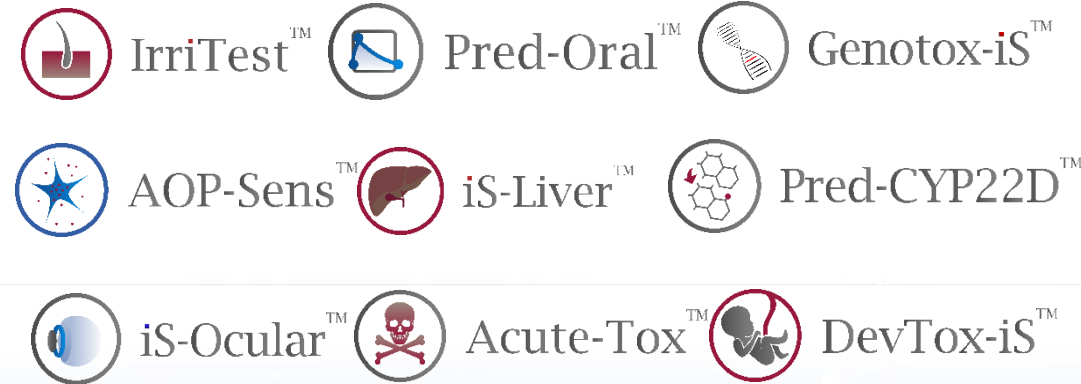
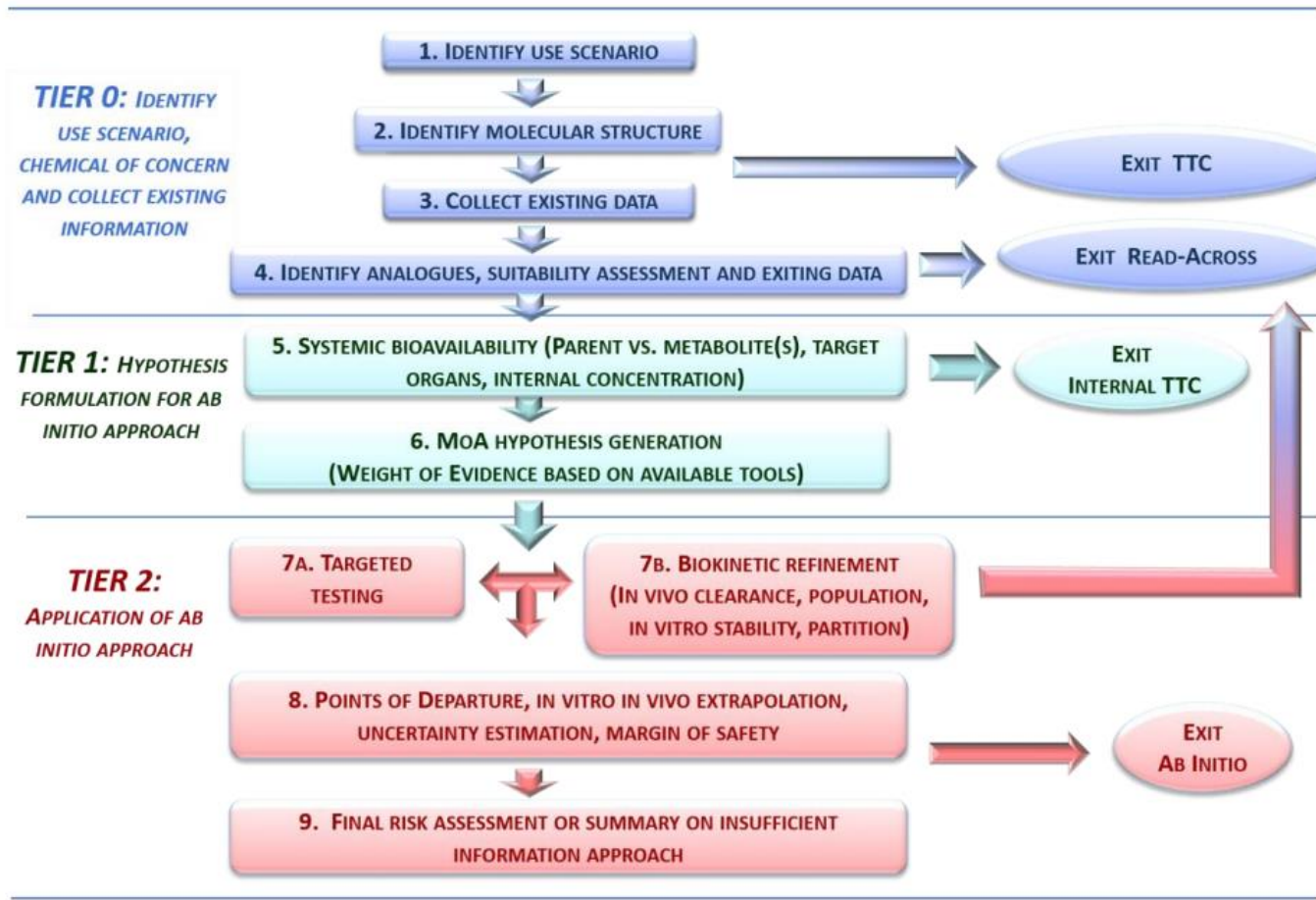
quimioteca com 7603 substâncias avaliadas para mutagenicidade *in vitro*.

Adverse Outcome Pathways (AOPs)

Constructo que descreve a cadeia sequencial de eventos-chave relacionados a um desfecho toxicológico (efeito adverso)



New Generation Risk Assessment (NGRA)



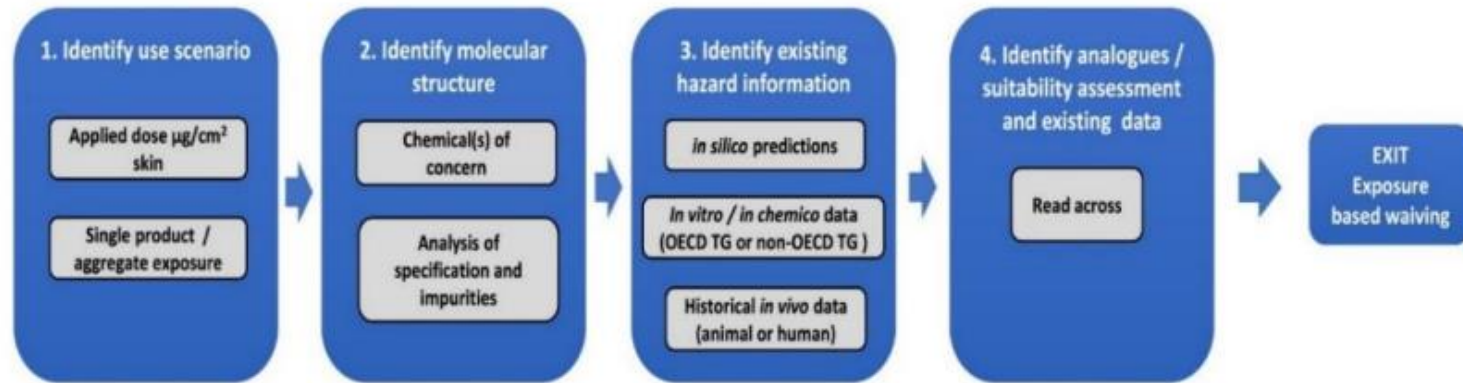
- ▶ Avaliação de características FQ, biodisponibilidade, metabolismo
- ▶ Avaliação de toxicidade sistêmica e para órgãos-alvo
- ▶ Avaliação para descartar potencial genotóxico, para proposição de limites baseados em toxicidade geral

- ▶ TTC: Classes I, II e III
- ▶ iTTC: 1µM

NGRA para sensibilização cutânea

Tier 0

Identify use scenario, chemical of concern and existing information



Tier 1

Hypothesis generation; how will data be used in risk assessment?



Tier 2

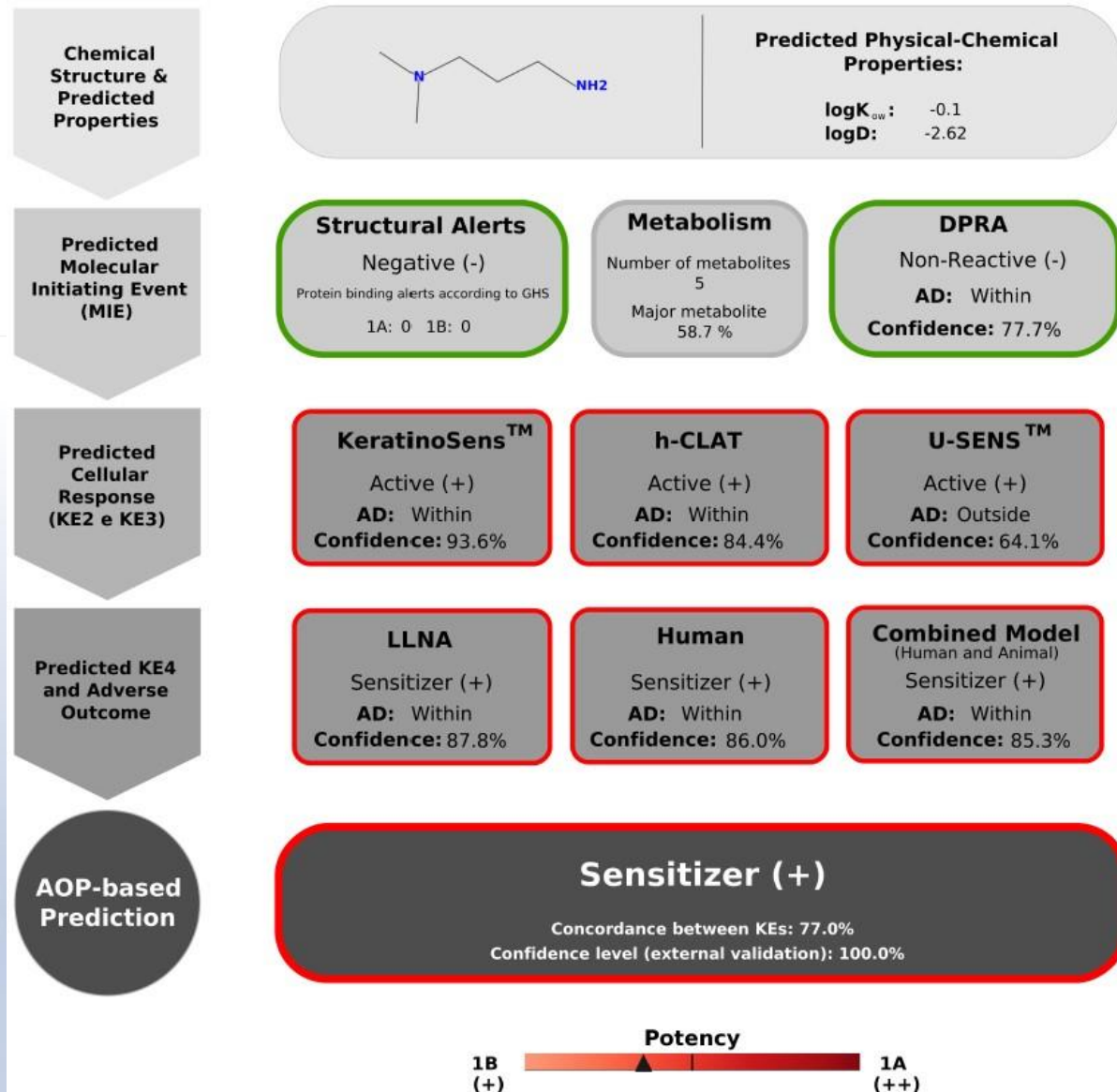
Risk assessment





AOP-SensTM

- Modelo *in silico* “AOP-based” for NGRA e Defined approaches on skin sensitisation (DASS)
- Avaliação de dados físico-químicos
- Verificação de alertas na estrutura e nível de preocupação de metabólitos previstos (eventuais pró-haptenos)
- Predição de cada um dos eventos-chave e desfecho de sensibilização
- Estimativa do peso de evidência (WoE) através de Key Events Relationships
- Estimativa de potência de sensibilização (Classes do GHS) que podem ser utilizadas para estimativas da DST (dermal sensitization threshold)
- Dados de análogos para RAX



Aplicando na prática

Name:

(R)(+)Limonene

CAS:

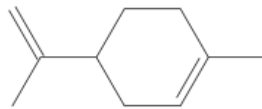
5989-27-5

SMILES:

CC1=CCC(CC1)C(=C)C

logK_{ow}:

3.31

**Name:**

1,2-Dibromo-2,4-dicyanobutane (MDGN)

CAS:

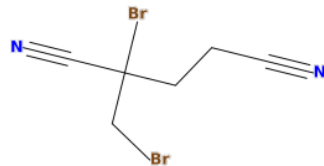
35691-65-7

SMILES:

C(#N)C(Br)(CC#N)CBr

logK_{ow}:

2.34

**Name:**

Isopropanol

CAS:

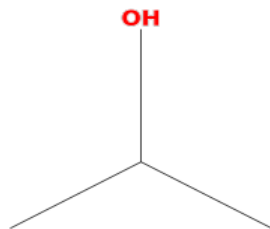
67-63-0

SMILES:

CC(C)O

logK_{ow}:

0.39

logD:

Perspectivas para desenvolvimento e aplicação regulatória no Brasil?

- ▶ **Diálogos e capacitação:** diálogos mais diretos, CPs e atualização das gerências de órgãos reguladores sobre estes novos métodos, práticas e abordagens;
- ▶ **Atualização regulatória:** Ausência de guias locais e normativas sobre avaliação de segurança atualizadas de acordo com avanços internacionais
- ▶ **Projetos de inovação entre empresas:** co-desenvolvimento de novos modelos *in silico*, estratégias NGRA, DAs e IATAs com foco em endpoints relevantes

Qual nível de atualização regulatória e inovação?



Nossos agradecimentos



Obrigado pela atenção!

carlos@altox.com.br

