European Commission

The EURL ECVAM Curated Database of Genotoxicity & Carcinogenicity Results
- Opportunities for Application in Regulatory Science -

Carcinogenesis Specialty Section (CSS) and Computational Toxicology Specialty Section (CTSS) Joint Webinar
14 December 2021

Carcinogenesis SOT Specialty Section of the Society of Toxicology

Federica Madia, PhD

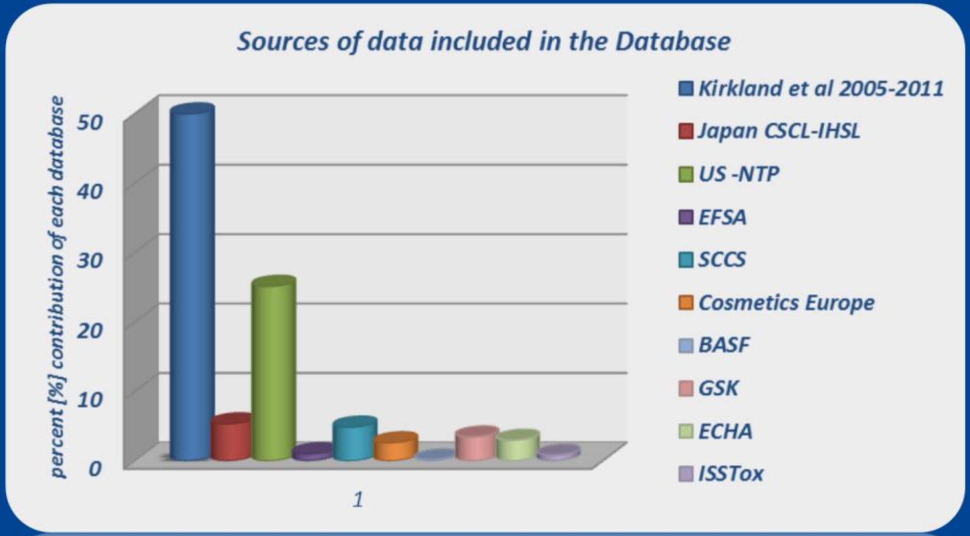
European Commission, JRC, Ispra ITALY

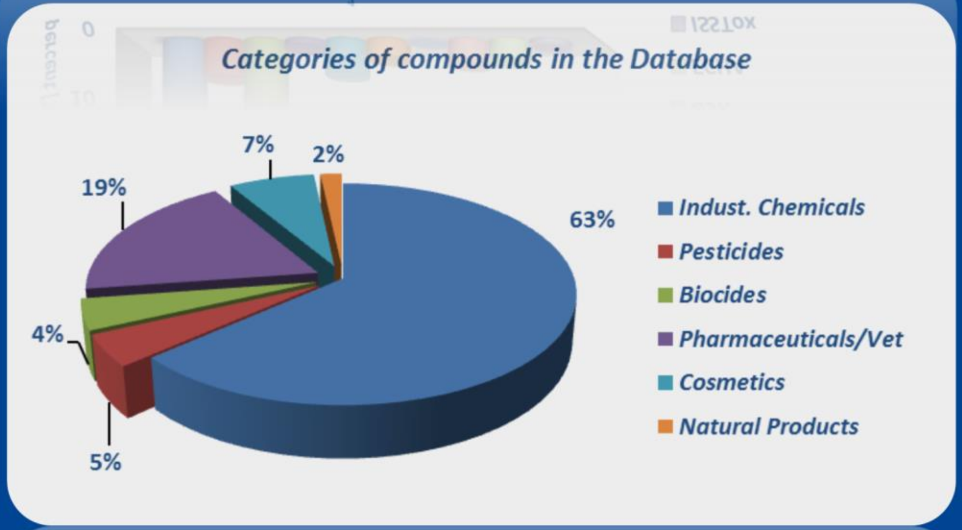
Joint Research Centre

Background

- ✓ The bacterial reverse mutation test (Ames test) is the most commonly used genotoxicity test
- ✓ Primary component of the chemical safety assessment data required by regulatory agencies worldwide
- ✓ Within the *in vitro* genotoxicity test battery, it reveals DNA reactivity, and identifies substances that can produce gene mutations *via* different mechanisms
- ✓ Evaluating its predictivity for ***in vivo* genotoxicity** and **carcinogenicity**, when considered alone or in association with mammalian cell assays for chromosome damage and/or gene mutations, is extremely important.

Collection of chemicals with Ames positive results



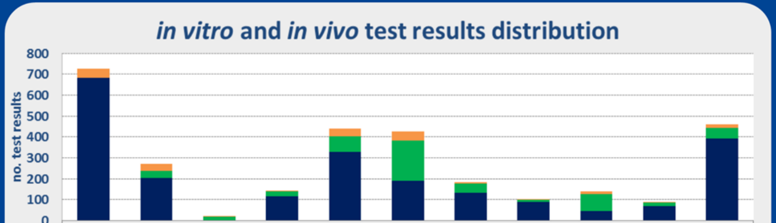


Mutation Research/Genetic Toxicology and Environmental Mutagenesis
Volumes 775–776, December 2014, Pages 55-68

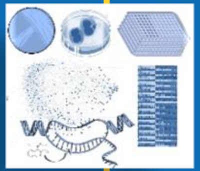
Can *in vitro* mammalian cell genotoxicity test results be used to complement positive results in the Ames test and help predict carcinogenic or *in vivo* genotoxic activity? I. Reports of individual databases presented at an EURL ECVAM Workshop

David Kirkland [a], Errol Zeiger [b], Federica Madia [c], Nigel Gooderham [d], Peter Kasper [e], Anthony Lynch [f], Takeshi Morita [g], Gladys Ouedraogo [h], Juan Manuel Parra Morte [i], Stefan Pfuhler [j], Vera Rogiers [k], Markus Schulz [l], Veronique Thybaud [m], Jan van Benthem [n], Philippe Vanparys [o], Andrew Worth [c], Raffaella Corvi [c]

> 700 substances



	Ames	MLA/Tk	Gene/ Hprt	MNvit	CAvit	MNviv	CAviv	TGR	UDSviv	DNAviv	Carc
E	44	31	1	2	38	45	8	1	16	2	17
-	0	36	19	22	75	191	43	12	78	19	53
+	682	203	0	118	328	192	134	89	47	68	392



Methodology

5 strains:
4 S. Typhimurium (TA)
and E.coli WP2

- ✓ "Overall Call" definition for each genotoxicity endpoint
- ✓ defined criteria for the reliability of each study and quality of data for those chemicals appearing in more than one source with different calls
- ✓ 4 categories: (+), (-), (E) and (I)

known chemical identity

(structure, purity, MW,CAS no)

clearly **negative** in the Ames test

full complement of strains as from OECD TG 471

valid *in vitro* and *in vivo* results for the genotoxicity endpoints

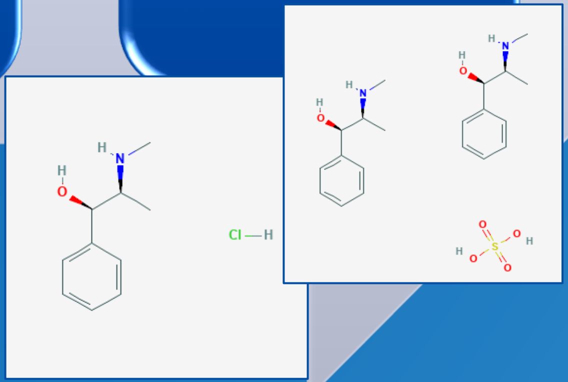
or for carcinogenicity

Combination free-bases and respective salts,

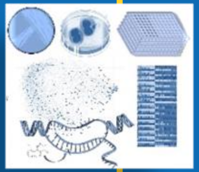
or R/S isomers

Curated expert review of all other endpoints

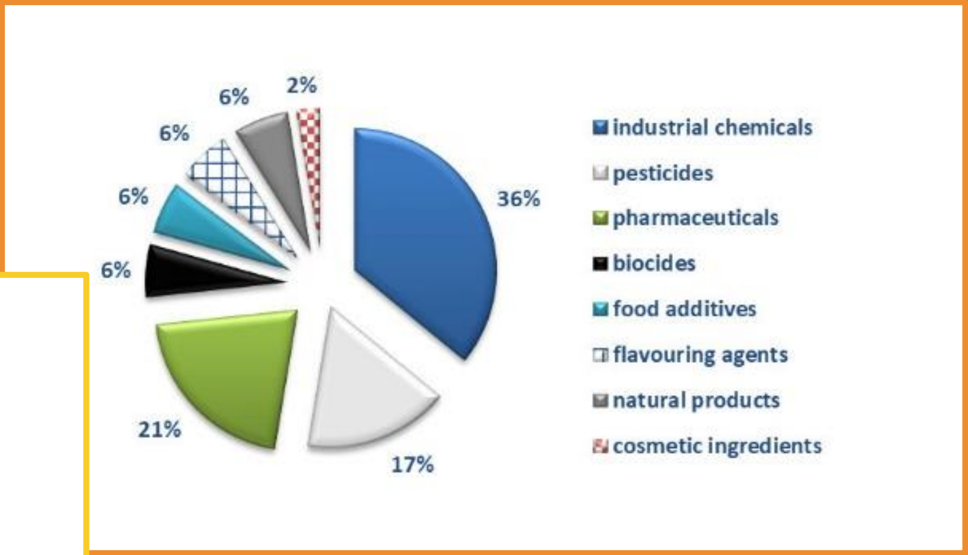
following defined criteria



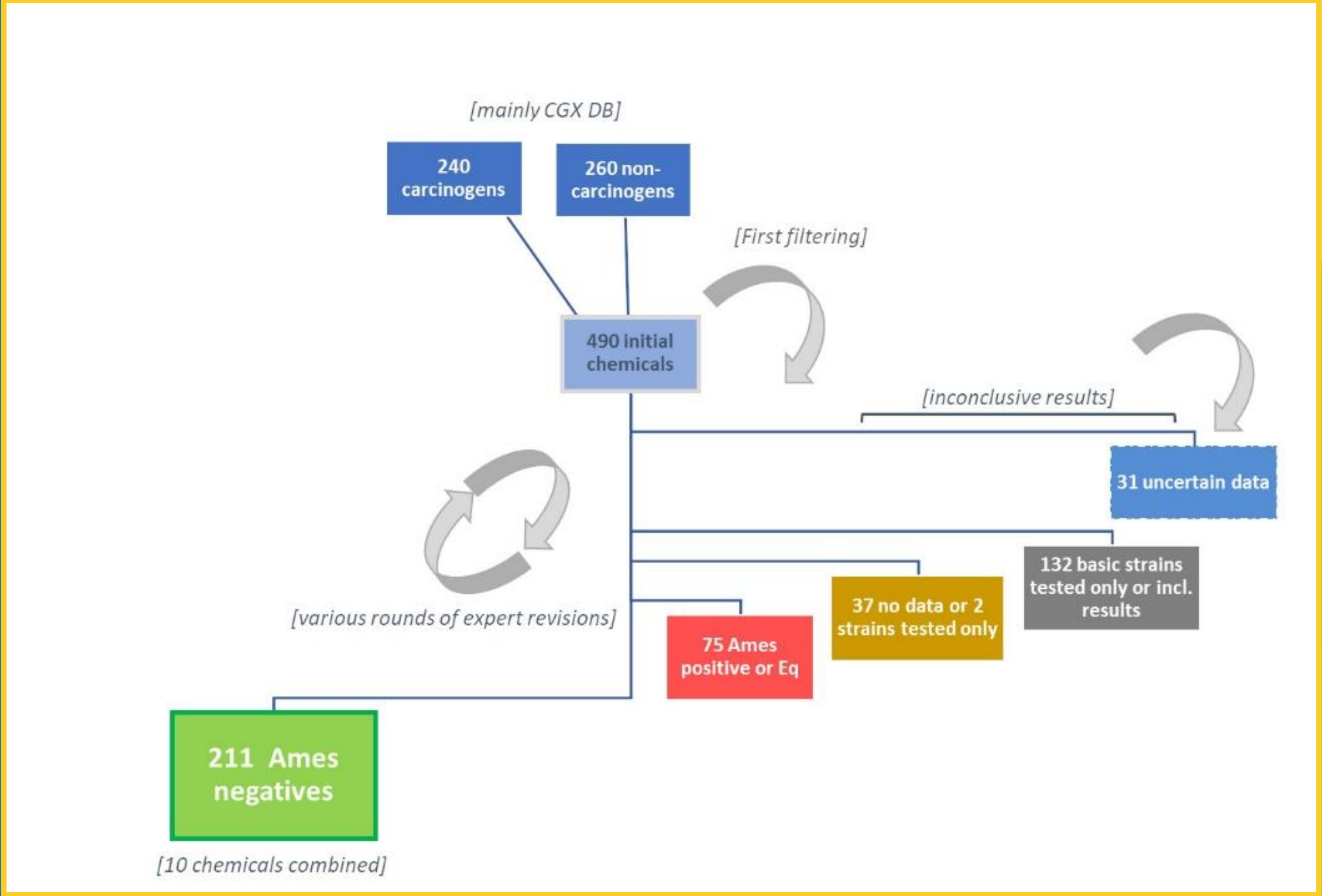
Reliability score >2 (ECHA)

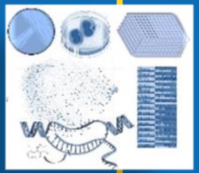


Data Review Process

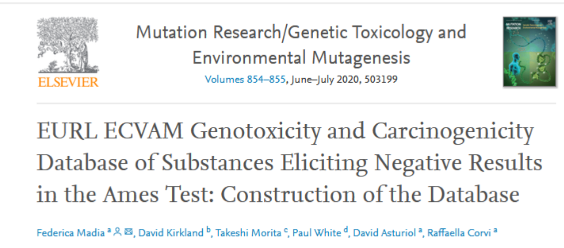


Curated expert review of available Ames test results





Test results distribution

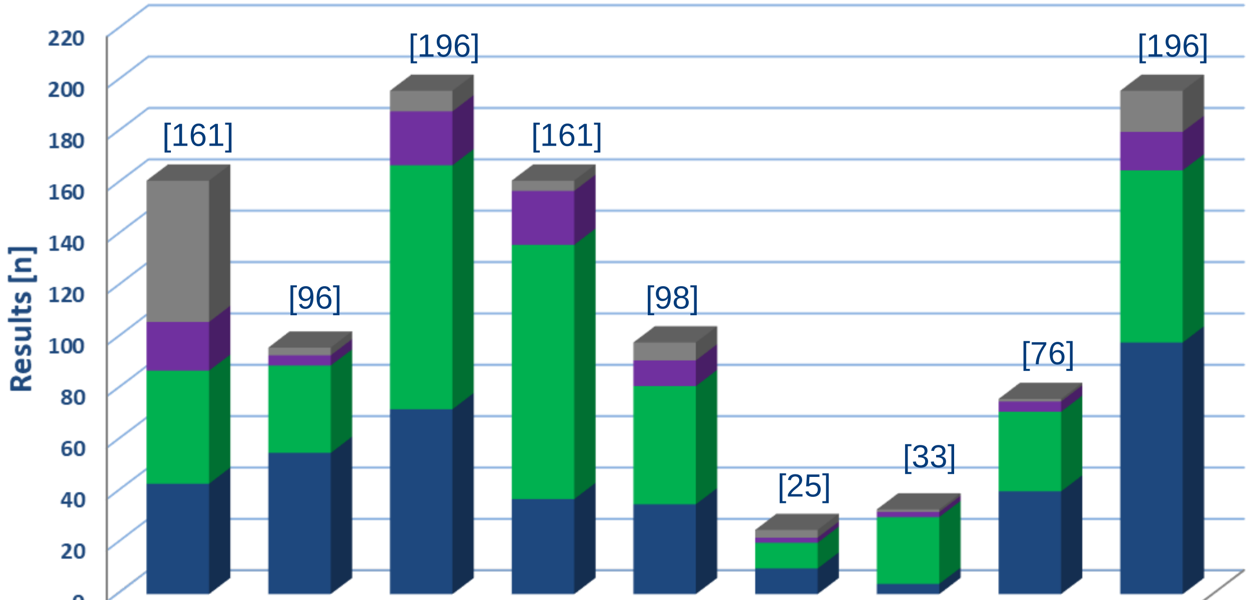


Mutation Research/Genetic Toxicology and Environmental Mutagenesis

Volumes 854–855, June–July 2020, 503199

EURL ECVAM Genotoxicity and Carcinogenicity Database of Substances Eliciting Negative Results in the Ames Test: Construction of the Database

Federica Madia [a], David Kirkland [b], Takeshi Morita [c], Paul White [d], David Asturiol [a], Raffaella Corvi [a]



	MLA/Tk/Hprt	MNvit	CAvit	MNviv	CAviv	TGR	UDSviv	DNAviv	Carc
[Inc]	55	3	8	4	7	3	1	1	16
[E]	19	4	21	21	10	2	2	4	15
[-]	44	34	95	99	46	10	26	31	67
[+]	43	55	72	37	35	10	4	40	98

Data were collected for the following tests:

genotoxicity

in vitro tests

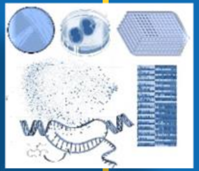
- Ames
- mouse lymphoma *Tk* [MLA] or *Hprt*
- micronucleus [MN]
- chromosome aberration [CA]

in vivo tests

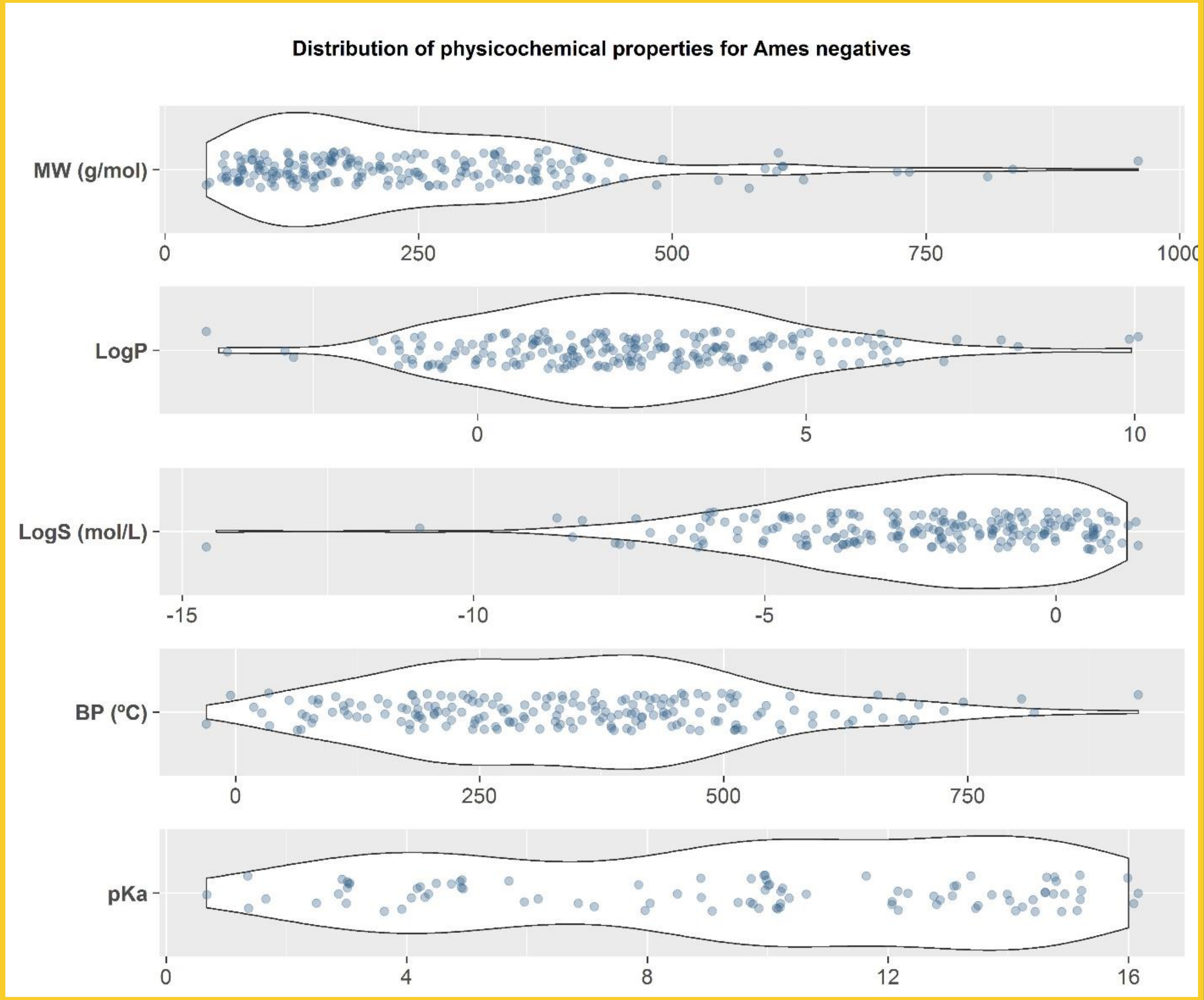
- micronucleus [MN]
- chromosome aberration [CA]
- unscheduled DNA synthesis [UDS]
- transgenic models,
- DNA breakage [Comet & alkaline elution]

rodent carcinogenicity

European Commission



Preliminary characterization: *physchem properties*

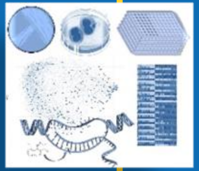


Most of Ames negatives are:

- ✓ rather small: with MWs below 500 g/mol; most of human drugs have MW<500
- ✓ are bioavailable: with logP values between -5 and 5. The former indicates high water solubility, the latter high octanol solubility
- ✓ not highly insoluble: the majority of substances have a solubility higher than 10-2 mol/L
- ✓ and mostly non-ionisable substances, as only 25 substances have pKa values below 7.0 (pKa predictions for 93 substances)

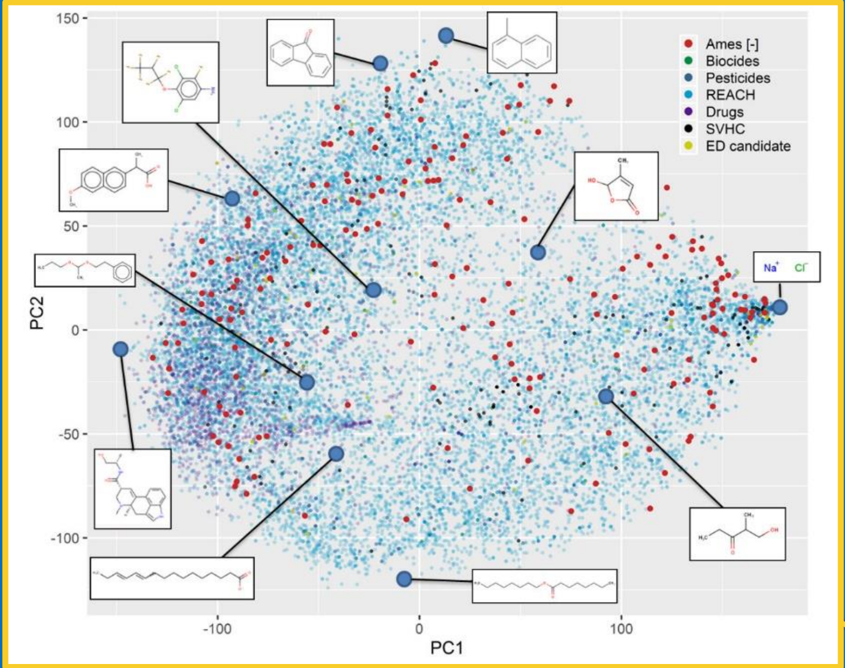
predicted with OPERA: https://jcheminf.biomedcentral.com/articles/10.1186/s13321-018-0263-1

European Commission

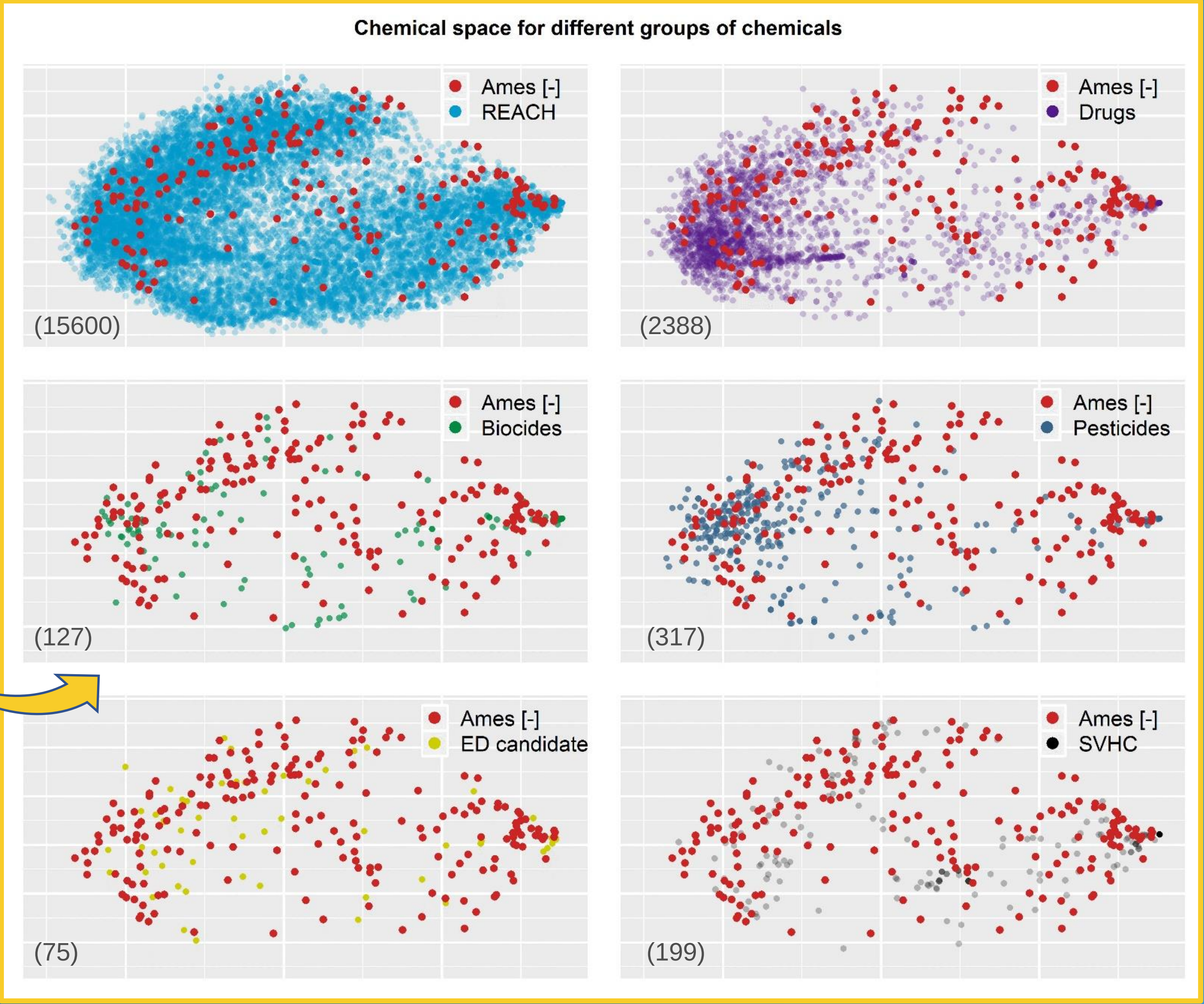


Preliminary characterization: *chemical space*

exploring the space of Ames negatives



Axes and positions of the substances correspond to the first two principal components of the similarity matrix of the substances built using the RDKit



An open access Database

	Chemical	CAS No.	InChiKey	Smiles	LogP	AMES Overall	in vitro MCGM Overall	in vitro MN Overall	in vitro CA Overall	in vivo MN Overall	in vivo CA Overall	TGR Overall	in vivo UDS Overall	in vivo DNA damage Overall	Rodent Carcinogenicity Overall	IARC Classification	Other Tests & Notes
1	Acephate	30560-19-1	E-UHFFFAOYSA-N	)(NC(=O)C)SC	-0.850	[-]	[+]		[E]	[-]	[+]			[+]	[I]		
2	Acesulfame-K	55589-62-3	EI-UHFFFAOYSA-M	]S(=O)(=O)O1	-0.930	[-]	[-]		[-]	[-]	[-]			[E]	[-]		[-] UDS in vitro rat primary hepatocytes#Jeffrey and Williams 2000; [-] DNA binding, rat, in spleen & liver#ECHA Database
3	Acetaldehyde	75-07-0	F-UHFFFAOYSA-N	CC=O	-0.340	[-]	[+]	[+]	[+]	[+]	[+]			[+]	[+]	Group 2B	[+] in vivo DNA [illegible] human blood cells from abusers#[illegible] and Vaca 1995; #Brooks and
4	Acetamide	60-35-5	B-UHFFFAOYSA-N	CC(=O)N	-1.260	[-]		[+]									
5	Acetohexamide	968-81-0	N-UHFFFAOYSA-N	)NC2CCCCC2	2.440	[-]	[I]										
6	Acetaminophen	103-90-2	F-UHFFFAOYSA-N	Nc1ccc(O)cc1	0.460	[-]	[+]	[+]									
7	Acetonitrile (Ethyl nitr	75-05-8	K-UHFFFAOYSA-N	CC#N	-0.340	[-]	[-]										
8	Acetic acid	64-19-7	D-UHFFFAOYSA-N	CC(=O)O	-0.170	[-]	[+]										
9	Acetoxime	127-06-0	B-UHFFFAOYSA-N	CC(=NO)C	0.120	[-]	[-]										

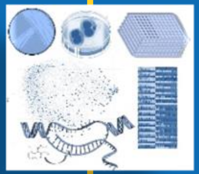
Database | Legend | Consulted Sources | References List

Ames Test

AMES CGX	AMES CGX references	AMES ISHL-CSL	AMES ISHL-CSL references	AMES NTP	AMES NTP references	AMES ECHA	AMES ECHA references	AMES EFSA	AMES EFSA references	AMES ISSTox	AMES Literature	AMES Overall
[-]	[-] mostly but [+] only >5000 ug/plate#Moriya et al 1983										[-] in TA98, TA100, TA1535, TA1537 and E. coli WP2#ECVAM Review 2016; [-] in TA98, TA100, TA1535, 1537 and E. coli	[-]
TC [-]	#Seifried et al 2006					[-]	#[-] in TA 98, TA 100, TA 1535, TA 1537, TA 1538 and E. coli WP2 uvrA, w/out S9				[-] in TA98, TA100, TA1535, TA1537 and E. coli WP2#ECVAM Review 2016	[-]
[-]		[-]	#[-] in TA98, TA100, TA1535, TA1537, TA1538 and E. coli WP2 uvrA, up to 5 mg/plate, w/out S9	[-]	[-] in TA98, TA100, TA1537, TA1535#Mortelmans et al 1986; [-] in TA100, TA102,	[-]/[+]	#[+] and [-] results reported in E. coli ([+] results superceded by robust later studies)				[-] in TA100, TA102, TA104#Dillon et al 1998; [-] in TA102#Aeschbacher et al 1989; [-] in E. coli WP2 uvrA#JETOC 1997	[-]
[-]				[-]	[-] in TA98, TA100, TA1535, TA1537 w/out S9#Haworth et al 1983	[-]	#[-] in TA97a, TA98, TA100, TA102, TA1535, up to 5000 ug/plate, w/out S9				[-] in TA98, TA100, TA1535, TA1537#Benedict et al 1977; [-] in TA102 and E. coli WP2, without S9; [-] in	[-]
[-]				[-]	[-] in TA97, TA98, TA100, TA1535, TA1537#Haworth et al 1983; [-] in E. coli WP2 w/out rat and							[-]
[-]				[-]	[-] in TA98, TA100, TA1535, TA1537, w/out S9#Haworth et al 1983; [-] in E. coli#IARC vol 50 1990						[-]#CPDB;#King et al 1979;#Dybing et al 1984;#Oldham et al 1986;#Haworth et al 1983;#Wirth et al 1980; [-] including TA102#Bartsch	[-]
TC [-]	[-] in TA98, TA100#Wu et al 2009			[-]	in TA97, TA98, TA100, TA1535, TA1537#Mortelmans et al 1986	[-]	[-] in TA98, TA100, TA1535, TA1537, TA1538 and E. coli WP2 uvr A, WP2 uvrA/pKM101, up to				[-] in TA98, TA100, TA1535, TA1537#Florin et al 1980; [-] in TA98, TA100#Schlegelmilch et al 1988	[-]
[-]	#Lambert et al 2005					[-]	[-] in TA92, TA94, TA98, TA100, TA1535, TA1537#Ishidate et al 1984; [-] in TA97, TA98, TA100, TA1535#Zeiger	[-]	[-] in TA98, TA100, TA1535, TA1537, TA1538 and E. coli WP2 and WP2 uvrA-, up to 1000ug/ml, w/out			[-]
[-]				[-]	[-] in TA97, TA100, TA1535, w/out S9	[-]	[-] in TA98, TA100, TA2637 and E. coli WP2 uvrA/pKM101, at up to 100 µmoles/ plate or a toxic level, by the					[-]
					[-] in TA97, TA98,		[-] in TA98, TA100,				[-]#Hashimoto and Tanii	

JRC Centre Data Catalogue
https://data.jrc.ec.europa.eu/dataset?q=ecvam&sort=sort_criteria+desc

DB progress



2014

The EURL ECVAM Genotoxicity and Carcinogenicity consolidated Database of Ames positives
(Kirkland et al 2014a, 2014b)

2014
Update of Addendum SCCS's Notes of Guidance SCCS/1532/14

2017
EFSA advice on the suitability of the *in vivo* UDS assay to follow-up positive results

2016
Evaluation and validation of several new genotoxicity tests & recommended list of chemicals

2017
Version 4
QSAR TOOLBOX
The OECD QSAR Toolbox for Grouping Chemicals into Categories

2018
OECD WG on DRP of miniaturised Ames tests

2019
HESI GTTC on analysis of *in vivo* follow-up from positive results

Section 4.5 Genotoxicity - WHO | World Health Organization

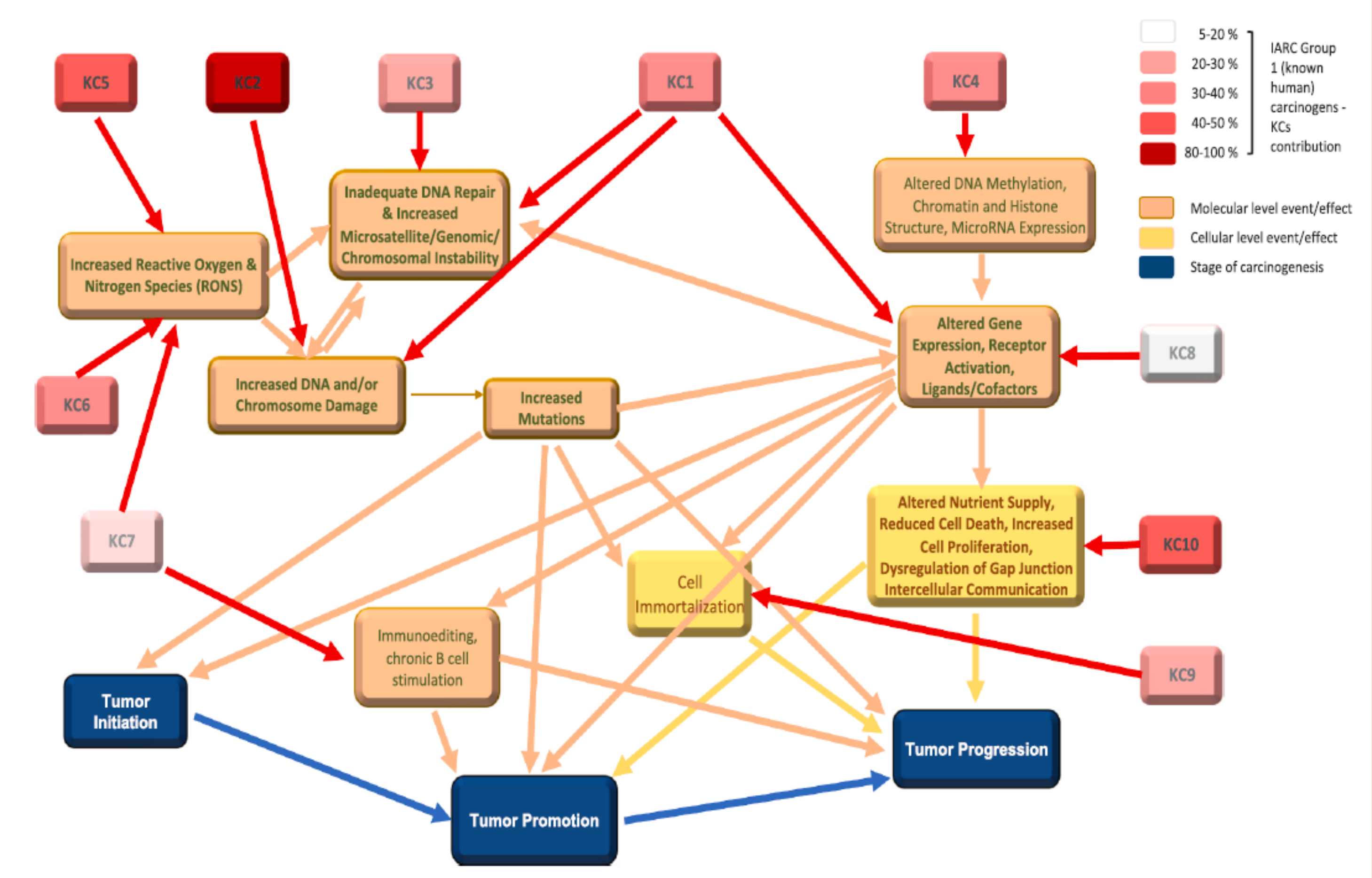
2020
Consolidated Database of Ames negatives

David Asturiol, EC-JRC
Raffaella Corvi, EC-JRC
Federica Madia, EC-JRC
Errol Zeiger, US
David Kirkland, Kirkland consulting, UK
Takeshi Morita, NITE, Japan
Paul White, Health Canada

Support the ICH M7 guideline on impurities recommended QSAR analyses

2021
Version 4.5
QSAR TOOLBOX
The OECD QSAR Toolbox for Grouping Chemicals into Categories

in silico approaches in carcinogenicity hazard assessment

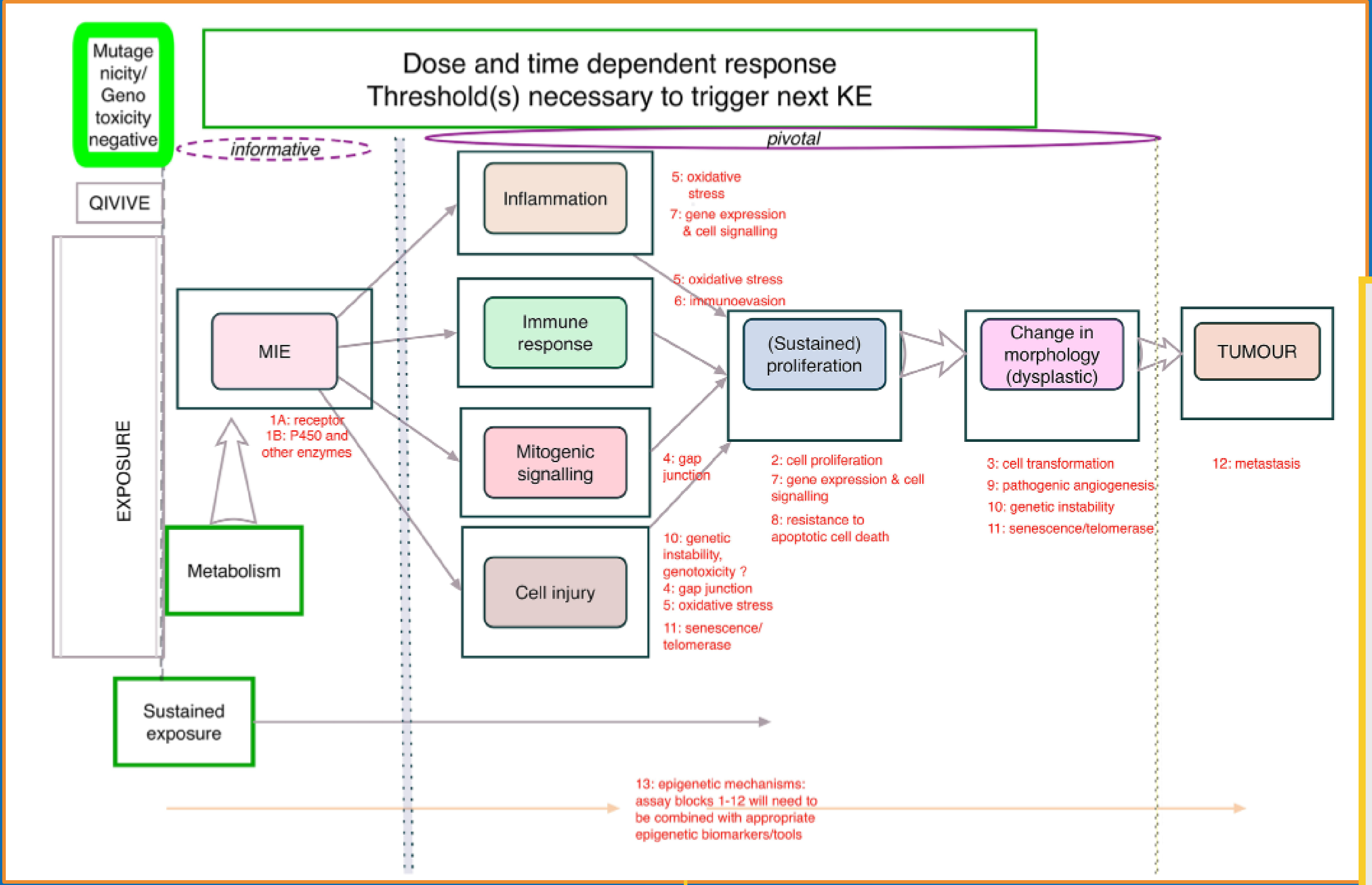


Tice et al 2021, https://doi.org/10.1016/j.comtox.2021.100191

Key Characteristics framework

Key Characteristic (KC)	First level, common molecular or cellular level event/effect among KCs	*In Silico* Methods: Availability	*In Silico* Methods: Used for making cancer-related regulatory decisions
KC1: Is Electrophilic or Can Be Metabolically Activated	KC2, KC3, KC8	Well-established for adduct formation, but otherwise limited	Yes, but limited to DNA reactivity
KC2: Is Genotoxic	KC1	Well-established	Yes
KC3: Alters DNA Repair Or Causes Genomic Instability	KC1	None	No
KC4: Induces Epigenetic Alterations		Limited	No
KC5: Induces Oxidative Stress	KC6, KC7	Not for cancer but for assay specific endpoints	No
KC6: Induces Chronic Inflammation	KC5, KC7	Not for cancer but could be developed for assay specific endpoints	No
KC7: Is Immunosuppressive	KC5, KC6	None	No
KC8: Modulates Receptor-Mediated Effects	KC1	Yes, for multiple nuclear receptors[1]	No
KC9: Causes Immortalization		Only for *in vitro* Syrian hamster embryo cell transformation	No
KC10: Alters cell proliferation, cell death, or nutrient supply		None	No

Development of IATA for non-genotoxic Carcinogens



Jacobs et al 2020, doi: 10.1007/s00204-020-02784-5

Block	Key hallmarks /characteristics (Mechanism/ mode of action)
1A	Receptor Binding and transactivation: Human Receptor
1B	P450 Induction and other enzymes
2	Cell Proliferation
3	Cell Transformation
4	Gap Junction
5	Indicators of Oxidative Stress
6	Immuno-evasion
7	Gene expression and cell signalling
8	Resistance to Apoptotic Cell Death
9	Pathogenic Angiogenesis and Neo-angiogenesis
10	Genetic Instability
11	Senescence/Telomerase
12	Metastasis
13	Epigenetic Mechanisms

News & Updates

https://www.getrevue.co/profile/comptox-newsletter

Computational Toxicology Newsletter

By CompTox Team

Hi there 👋 and Welcome to this little virtual corner ✨ Our aim is to curate peer-reviewed publications that showcase novel strategies or good case studies of in silico models useful in drug discovery or chemical safety assessment. With this, we hope to help keep our readers updated on Computat... Read more

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#2 · DEC 4, 2021

Computational Toxicology Newsletter - Issue #2

Quick summary:In vitro and in silico investigations of endocrine disruption induced by metabolites of plasticizers through glucocorticoid receptor Molecular docking Molecular dynamicsIn silico prediction of nuclear receptor binding to polychlorinated di

#1 · OCT 7, 2021

Computational Toxicology Newsletter - Issue #1

ToxAnalyzer: A user-friendly web tool for interactive data analysis and visual chemical compounds from the Comparative Toxicogenomics Database (CTD) Computational Toxicology by Ribeiro Rodriguez et al.✨ MotivationThe "Cor Toxicogenomics ...

https://estiv.org/in3/index.php

Computational Toxicology

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The in silico toxicology protocols initiative

Edited by Glenn Myatt, Kevin Cross, Candice Johnson

Last update 23 September 2021

In silico toxicology (IST) encompasses the use of computational models, based on different methodologies, to make predictions of the toxicity of a chemical. IST is being used to support an increasing number of applications for both regulatory purposes as well as to support industrial use cases. This increased usage is driven by several factors, including the desire to reduce animal testing and the need to increase the efficiency and overall safety of bringing new chemical products to market. The use of in silico assessment has in turn driven the development of IST best practices to ensure their usage is both transparent and defendable. Computational frameworks that incorporate such in silico practices will have a critical role in the expanding area of IST.

https://www.sciencedirect.com/journal/computational-toxicology/collection/10ZD1MJ9982?page-size=100&page=1

InSilicoInsider

https://insilicoinsider.blog/

Nicoleta Spinu, Ana Caballero, Susana Proenca, Liadys Mora Lagares & Andrew Worth

European Commission

Thank you for your Attention!

Any questions?

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