Modelli SAR per la predizione della genotossicità in vivo di sostanze chimiche: Revisione del modulo Structure Alerts for the in vivo micronucleus assay e aggiornamento della banca dati ISSMIC

Facoltà di Farmacia e Medicina

SAPIENZA
UNIVERSITÀ DI ROMA

Corso di Laurea Magistrale in Biotecnologie Farmaceutiche (LM-9)

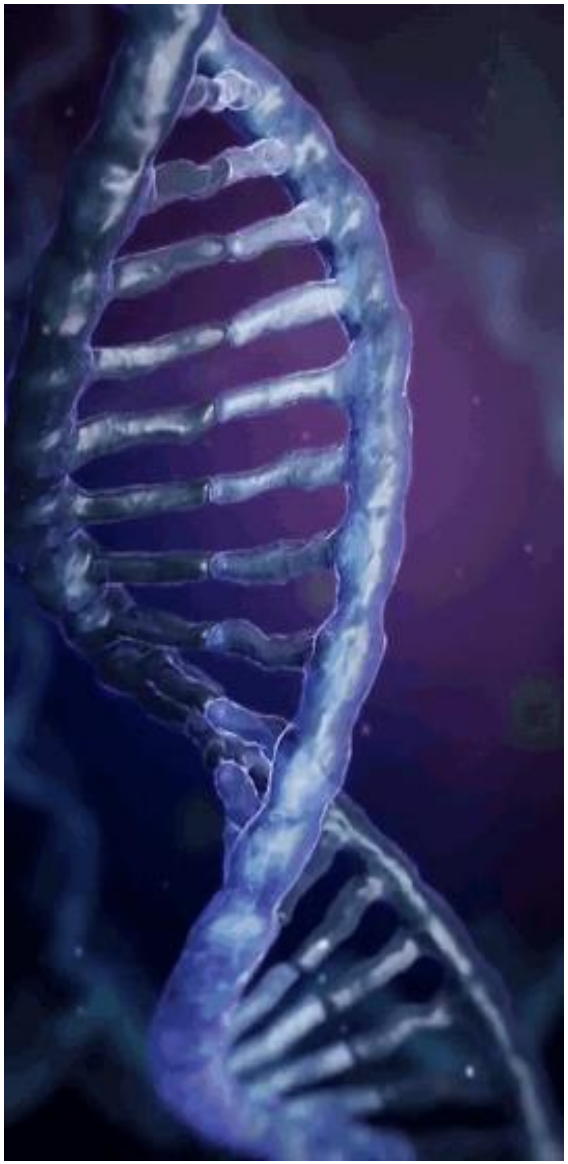
Tesi sperimentale in Tossicologia Computazionale

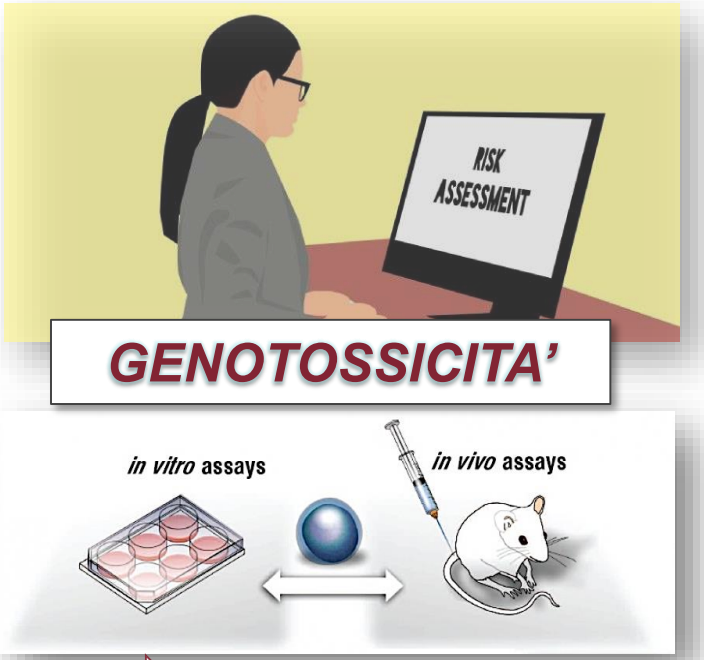
Anno accademico 2019/2020

Laureanda: Lorenza Troncarelli
Matricola: 1547454

Relatore: Prof. Rino Ragno
Correlatrice: Dott.ssa Cecilia Bossa

Valutazione della Genotossicità





Fase 1: Test in vitro

- Test di Ames
- Test di aberrazioni cromosomiche

✓

Fase 2 (Follow-up) Test in vivo

- **Test del Micronucleo**
- Test della Cometa
- Test di sintesi non programmata di DNA (UDS

?

Regolamento REACH (allegato XI)

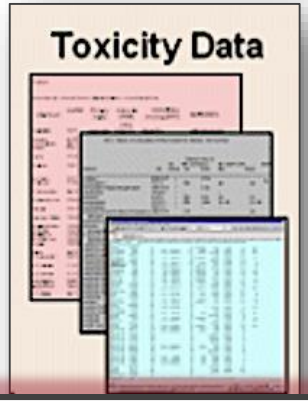
« LA SPERIMENTAZIONE NON E' SEMPRE NECESSARIA! »

Utilizzabile solo come estrema ratio

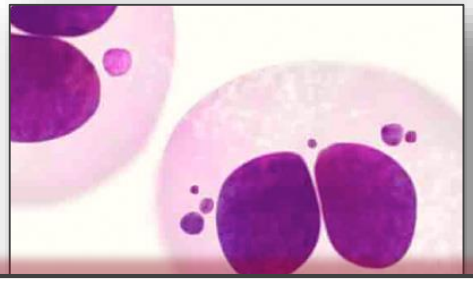
Uso di dati esistenti	• Raccolta e condivisione dei dati
Peso dell'evidenza	• Raccolta informazioni da tutte le fonti possibili (indipendenti)
Metodi in vitro	• Tessuti, organi, cellule isolati
Metodi in silico	• Read across e grouping • QSAR e SAR

ALLERTE STRUTTURALI

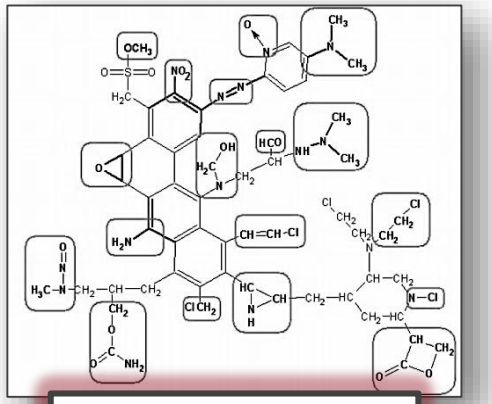
Profiler ISS (MN): ALLERTE STRUTTURALI



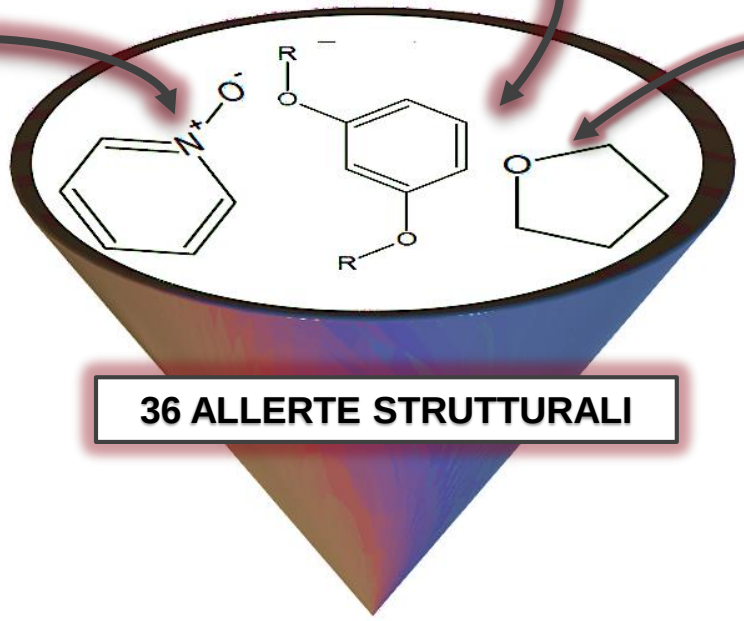
Dati di sostanze testate al micronucleo



Meccanismi d'azione per la formazione dei MN



Sottostrutture mutagene note (Ashby)



36 ALLERTE STRUTTURALI

SCREENING PRELIMINARE DI POTENZIALI SOSTANZE GENOTOSSICHE IN VIVO

Database ISSMIC

Che cos'è?

UN CHEMICAL RELATIONAL DATABASE

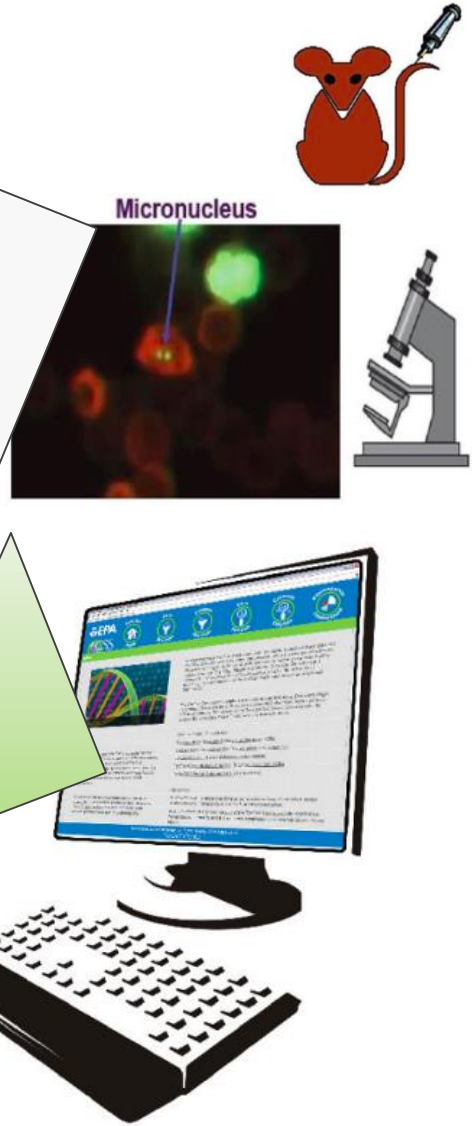
Che cosa contiene?

566 sostanze testate al micronucleo in vivo

Trattamento dei dati?

4 tipologie di Overall Value:

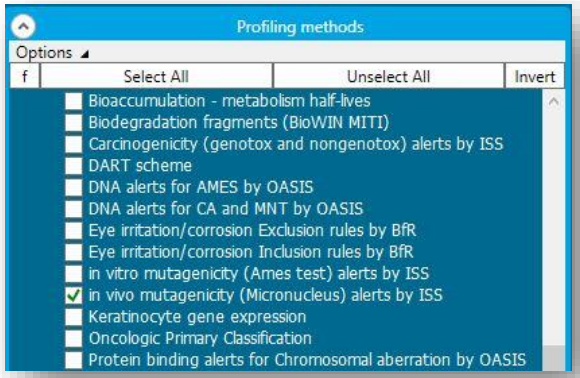
- **190 sostanze Positive**
- **97 sostanze Negative** → ***Toxicity Biomarker***
- **48 sostanze Equivoche**
- **231 sostanze Inconclusive**



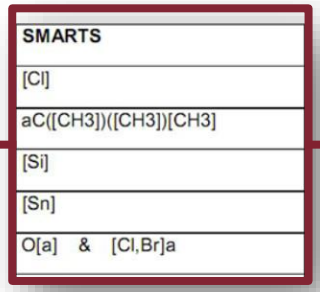
IMPLEMENTAZIONE INFORMATICA

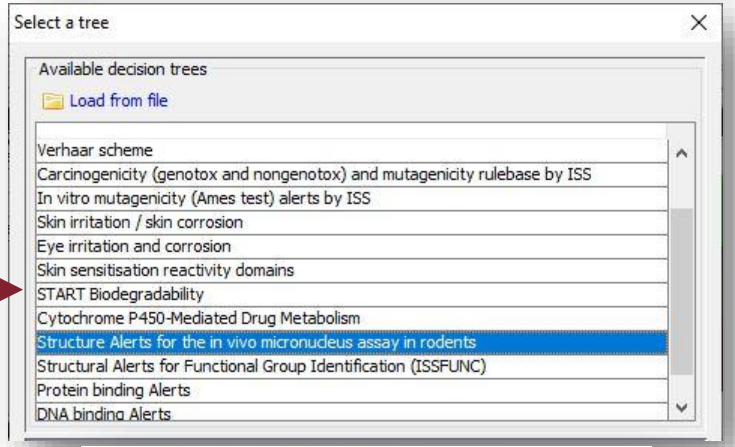
QSAR TOOLBOX

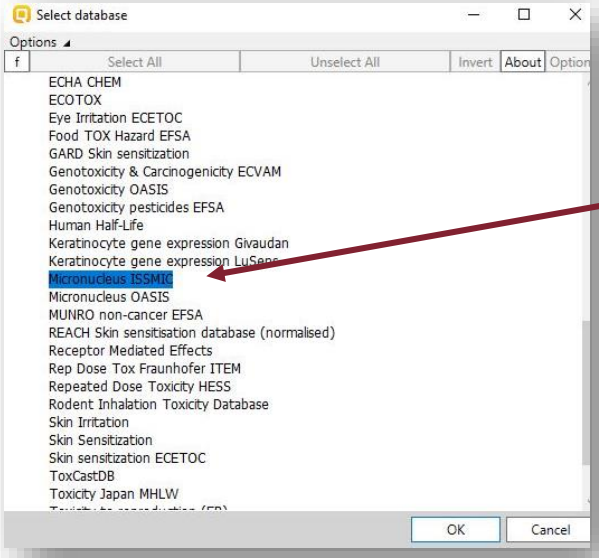
Toxtree



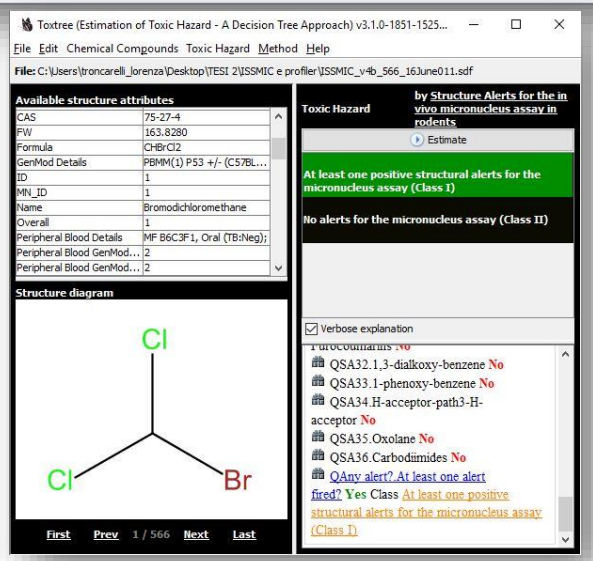
PROFILER ISS (MN)



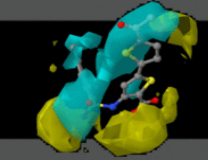




DATABASE ISSMIC



FLUSSO DI LAVORO



REVISIONE DEL PROFILER ISS (MN)

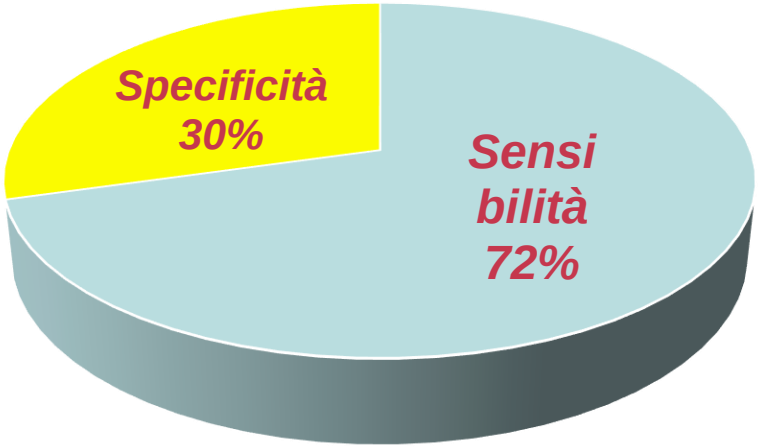
- STATO DELL'ARTE DEL PROFILER ISS (MN)
  - TOXTREE (v. 3.1.0)
- PREDITTIVITA' POSITIVE DELLE ALLERTE
  - TOXTREE (v. 3.1.0) VS QSAR TOOLBOX (v. 4.3.1)
- CASO STUDIO SA 32
  - NUOVA DEFINIZIONE DELL'ALLERTA
- CASO STUDIO SA 10 E SA 28
  - DEFINIZIONI ALLERTE CORRETTE
- PROFILER ISS (MN) VS PROFILER OASIS (MN)
  - Micronucleus OASIS
- NUOVA ALLERTA: BENZIMIDAZOLO
  - SOSTANZE BENZIMIDAZOLICHE

AGGIORNAMENTO ISSMIC

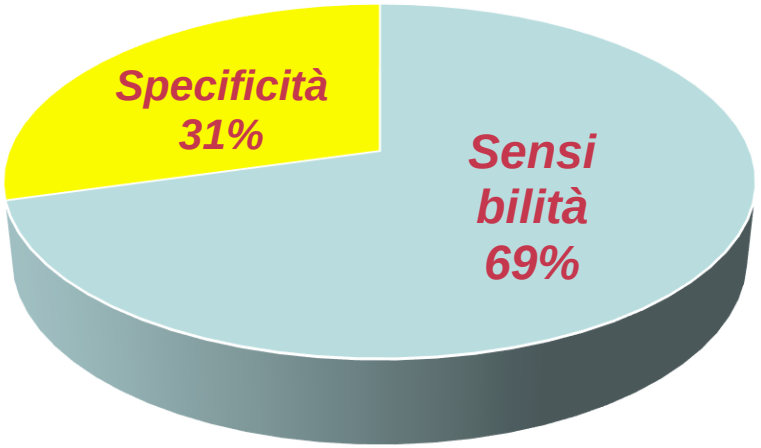
- RICERCA NUOVE SOSTANZE
  - Micronucleus OASIS
  - CGX ECVAM
- 120 SOSTANZE
  - SOSTANZE BENZIMIDAZOLICHE
- ANALISI DELLE FONTI DI LETTERATURA (MORITA ET AL. 1997)
  - **51 SOSTANZE NUOVE**

Performance predittiva del Profiler in ISSMIC

TOXTREE v.3.1.0



QSAR TOOLBOX v.4.3.1



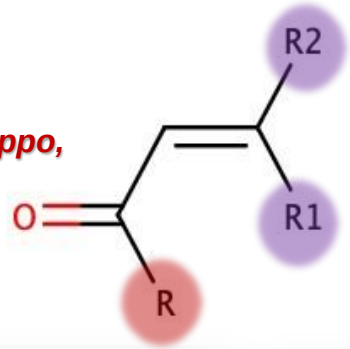
Calcolo delle predittività' positive delle singole allerte

	TOXTREE			*QSAR TOOLBOX*		
SA (*PROFILER* ISS MN)	SOSTANZE CON SA	POSITIVE AL MICRONUCLEO	PREDITTIVITA' POSITIVA (%)	SOSTANZE CON SA	POSITIVE AL MICRONUCLEO	PREDITTIVITA' POSITIVA (%)
SA 1	0	0		0	0	
SA 2	4	3	75 %	4	3	74 %
SA 3	1	0	0	1	0	0
SA 4	3	3	100 %	3	3	100 %
SA 5	4	4	100 %	4	4	100 %
SA 6	0	0		0	0	
SA 7	13	12	92 %	13	12	92 %
SA 27	6	4	67 %	6	4	67 %
SA 28	32	26	81 %	26	20	77 %
SA 28 bis	4	2	50 %	4	2	50 %
SA 28 ter	1	0	0	1	0	0

RISULTATI: CASO STUDIO SA 10

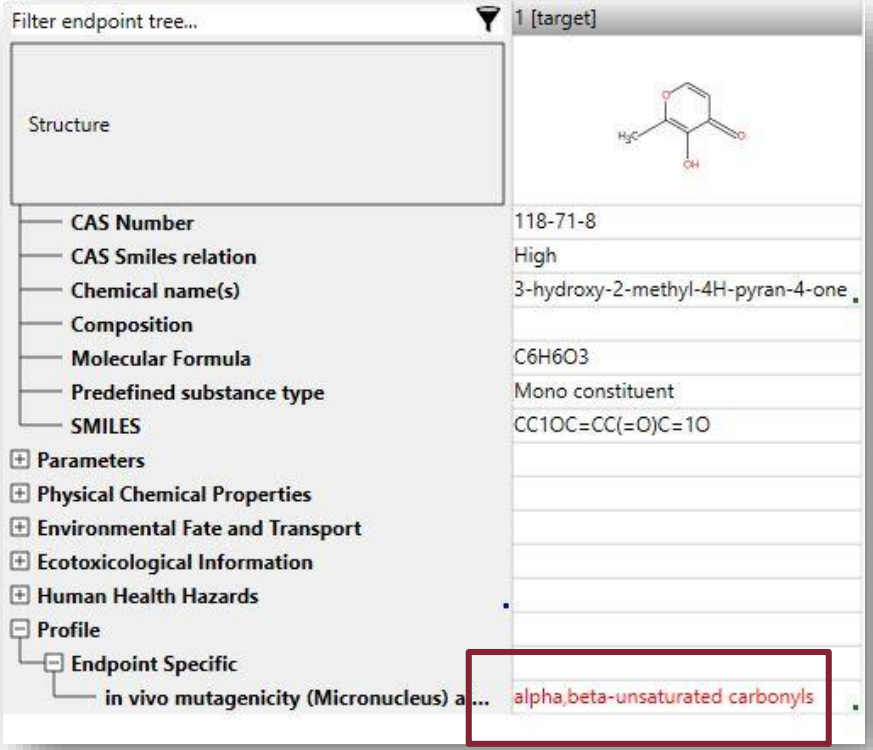
α,β-unsaturated carbonyls

R: qualsiasi atomo/gruppo, tranne OR, OH

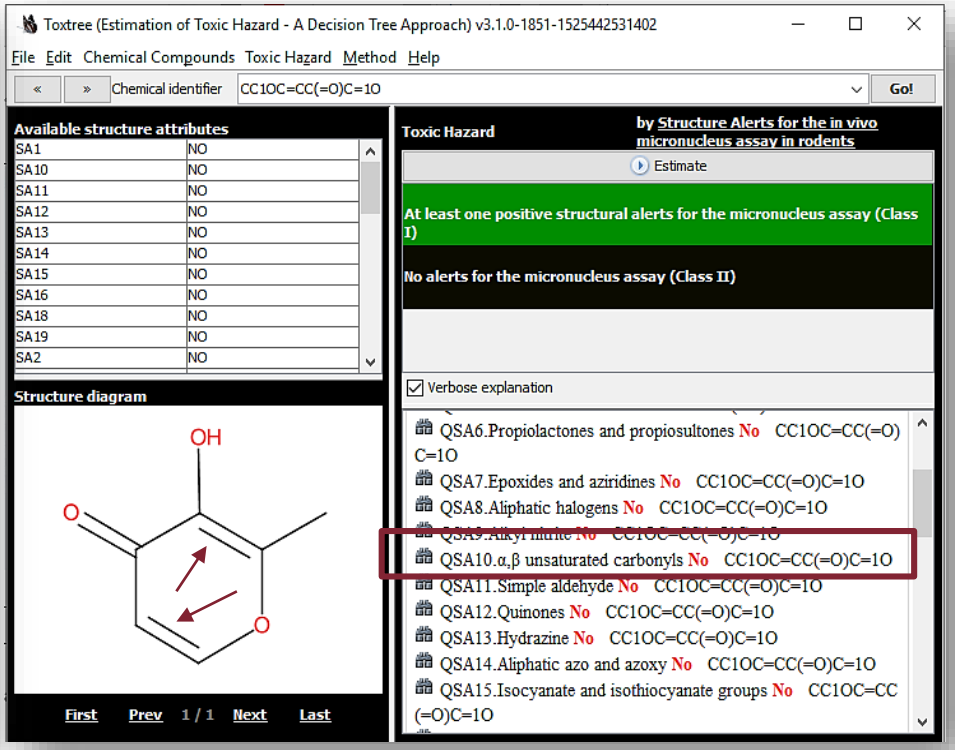


R1 e R2: qualsiasi atomo / gruppo, tranne le catene alchiliche con C> 5 o anelli aromatici

QSAR TOOLBOX

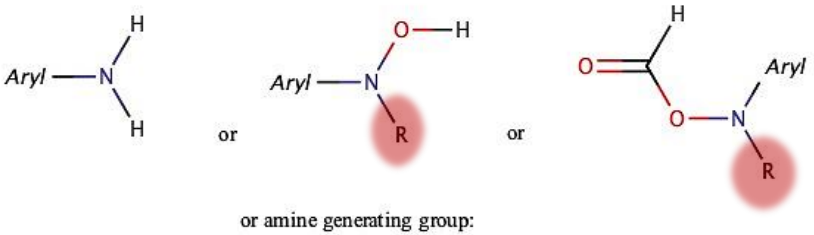


TOXTREE



RISULTATI: CASO STUDIO SA 28

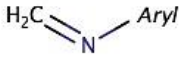
Primary aromatic amine, hydroxyl Amine and its derived esters



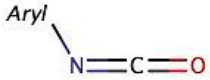
R: qualsiasi atomo/gruppo eccetto:

- ***composti di-sostituiti o con un acido carbossilico in orto come sostituente***
- ***composti con un gruppo solfonico (-SO_3H) sullo stesso anello in cui si trova il nitro gruppo***

QSAR TOOLBOX



or



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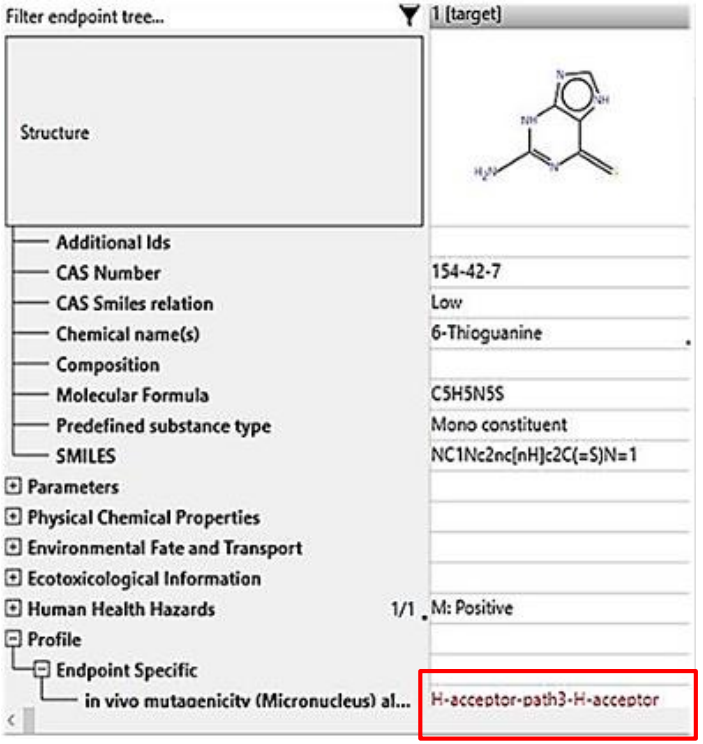
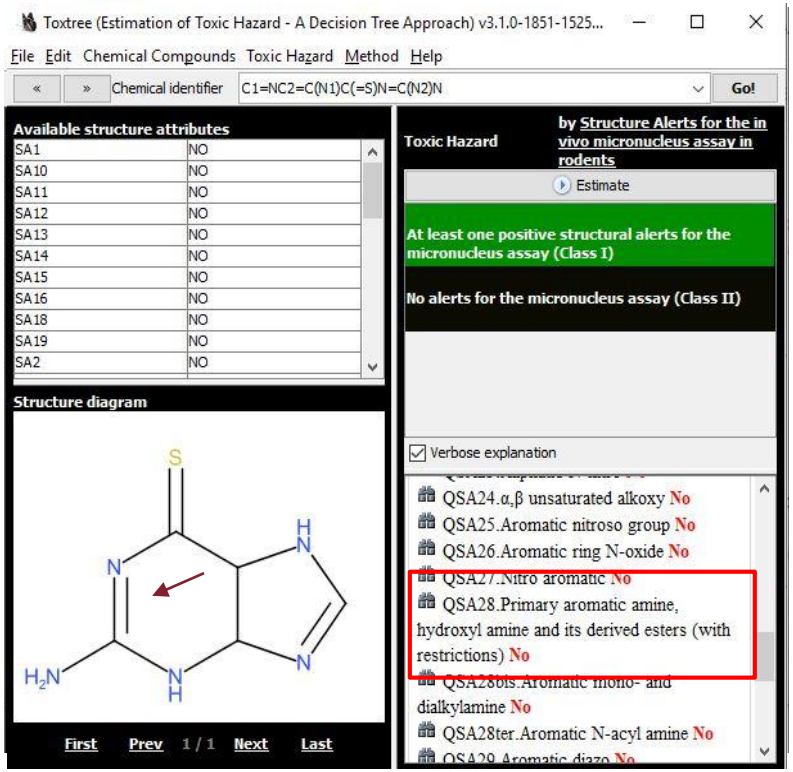
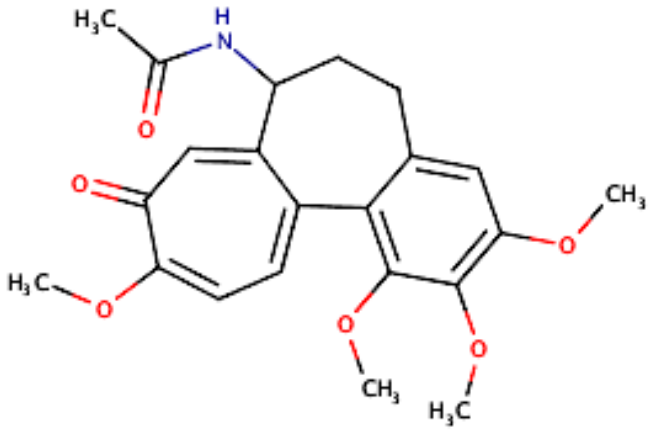
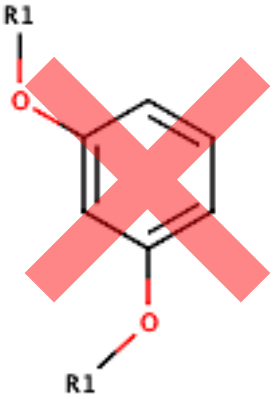


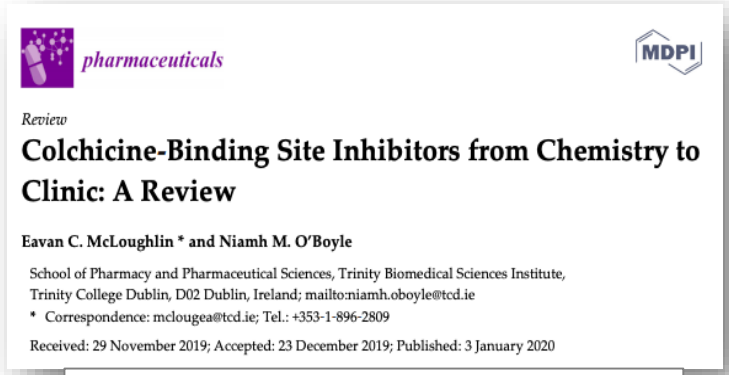


RISULTATI: CASO STUDIO SA 32



COLCHICINA





pharmaceuticals

MDPI

Review

Colchicine-Binding Site Inhibitors from Chemistry to Clinic: A Review

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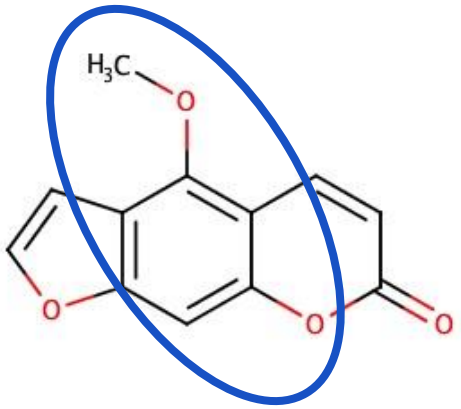
Received: 29 November 2019; Accepted: 23 December 2019; Published: 3 January 2020

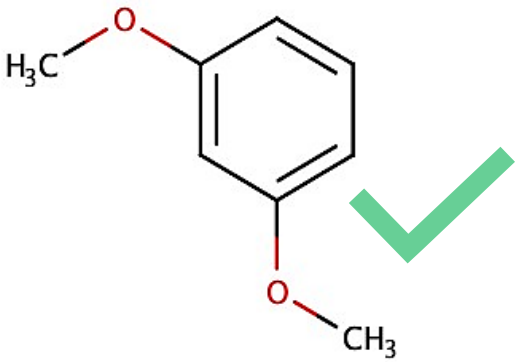
IMPORTANZA DEI GRUPPI METOSSI NELL'ANELLO A DELLA COLCHICINA



BERGAPTENE

DEFINIZIONE ATTUALE:
1,3-DI-ALCOSSIBENZENE

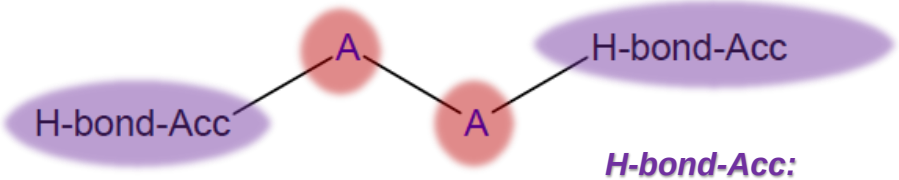
RAGGIO D'AZIONE DELL'ALLERTA TROPPPO LARGO!



NUOVA DEFINIZIONE:
1,3-DI-METOSSIBENZENE

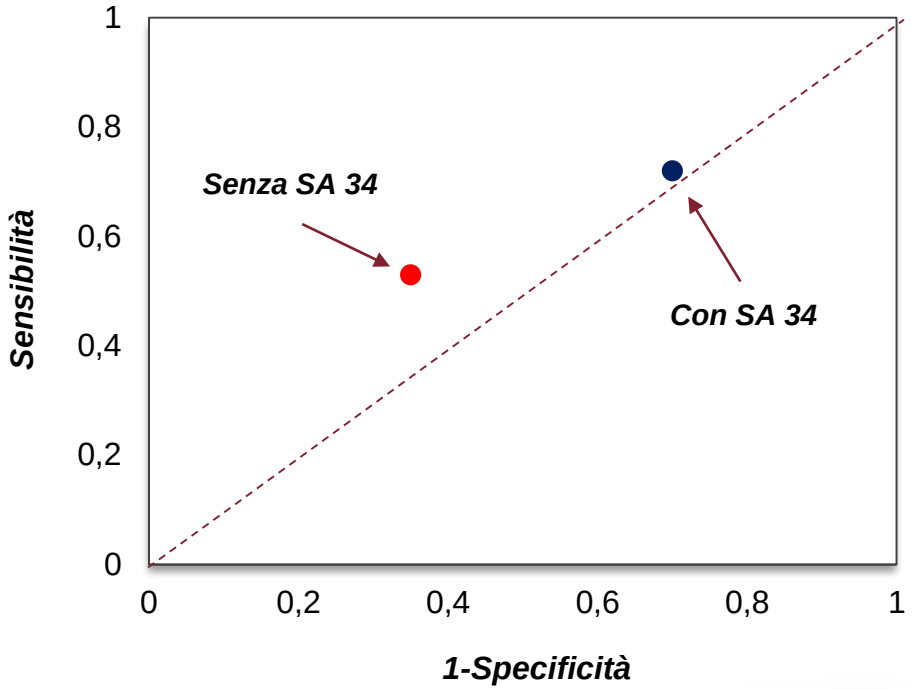
RISULTATI: CASO SA 34

H-acceptor-path3-H-acceptor



A: qualsiasi atomo tranne H

H-bond-Acc: qualsiasi atomo accettore di legami idrogeno



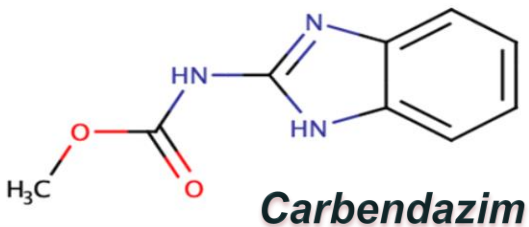
Problemi di bassa specificita'

Ricerca di nuove ipotesi meccanicistiche

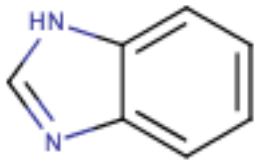
Studio chemio-informatico

QSAR TOOLBOX

RISULTATI: NUOVA SA?



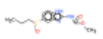
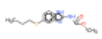
Carbendazim



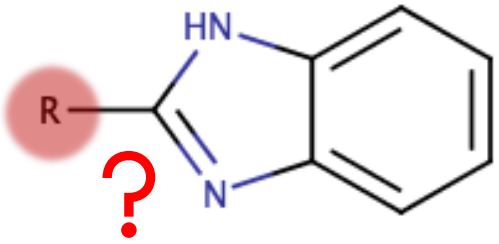
Benzimidazoli

Inibiscono la polimerizzazione della tubulina (sito di legame non identificato)

Filter endpoint tree...

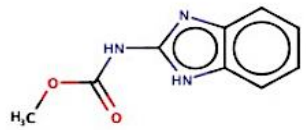
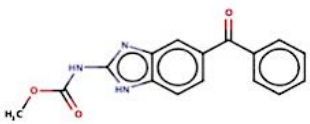
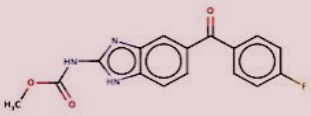
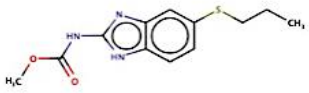
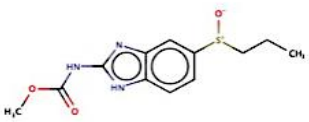
	1	2	3	4	5	6	7	8	9
Structure									
Structure info									
Additional Ids	EC Number:2342...		EC Number:2506...		EC Number:2057...	EC Number:2462...	EC Number:2594...	EC Number:2417...	
CAS Number	10605-21-7	73590-58-6	31431-39-7	54029-12-8	148-79-8	24370-25-0	54965-21-8	17804-35-2	105650-23-5
CAS Smiles relation	High	Moderate	High	Low	High	High	High	High	Moderate
Chemical name(s)	1h-benzimidazol-...	Omeprazole"""	Carbamic acid, (...	Albendazole oxide	1H-Benzimidazol...	1-(1H-benzimida...	[5-(Propylthio)-1...	[1-[(butylamino)...	2-Amino-1meth...
Composition									
Molecular Formula	C9H9N3O2	C17H19N3O3S	C16H13N3O3	C12H15N3O3S	C10H7N3S	C8H8N4O	C12H15N3O2S	C14H18N4O3	C13H12N4
Predefined substance type	Mono constituent	Mono constituent	Mono constituent	Mono constituent	Mono constituent	Mono constituent	Mono constituent	Mono constituent	Mono constituent
SMILES	COC(=O)Nc1nc2c...	COc1ccc2[nH]c(n...	COC(=O)Nc1nc2...	CCC[S+]([O-])c1c...	c1ccc2[nH]c(nc2...	NC(=O)Nc1nc2c...	CCCSc1ccc2[nH]...	CCCCNC(=O)n1c...	Cn1c(N)nc2ncc(c...
Parameters									
Physical Chemical Properties									
Environmental Fate and Transport									
Ecotoxicological Information									
Human Health Hazards									
Genetic Toxicity									
in Vivo									
Micronucleus Assay 18/34	M: Positive	M: Positive	M: Positive	M: Positive	M: Positive	M: Positive	M: Positive	M: Positive	M: Positive
Profile									
Endpoint Specific									
in vivo mutagenicity (Micronucleus) al...	H-acceptor-path...	H-acceptor-path...	H-acceptor-path...	H-acceptor-path...	H-acceptor-path...	H-acceptor-path...	H-acceptor-path...	H-acceptor-path...	H-acceptor-path...

RISULTATI: NUOVA SA?



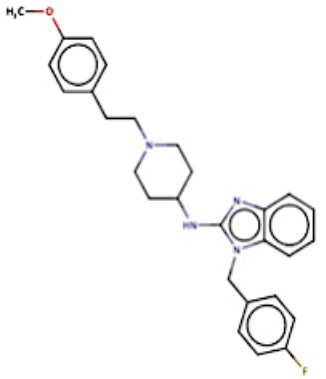
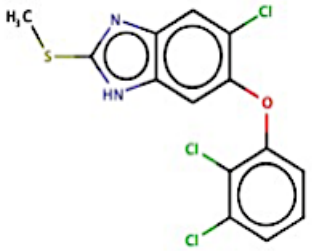
1) IPOTESI:

R = CARBAMMATO →
MAGGIORE STABILITA' DI LEGAME

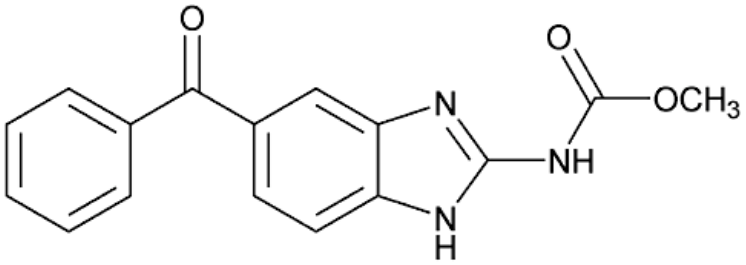
CAS	Nome	Struttura	Risultato MN
10605-21-7	Carbendazim		+
31431-39-7	Mebendazolo		+
31430-15-6	Flubendazolo		-
54965-21-8	Albendazolo		+
54029-12-8	Albendazolo Sulfossido		+

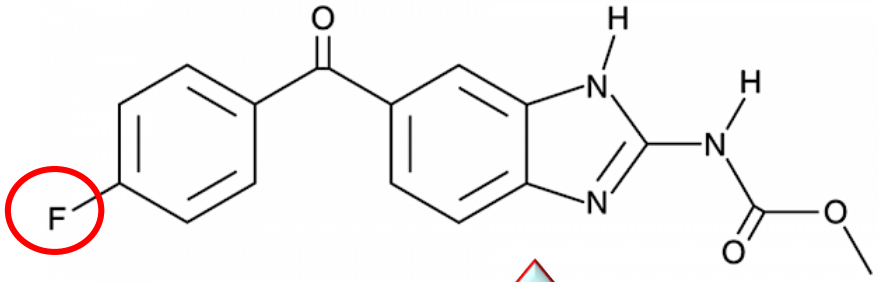
2) IPOTESI:

R = GRUPPI VOLUMINOSI →
POSSIBILE INGOMBRO STERICO

CAS	Nome	Struttura	Risultato MN
68844-77-9	Astemizolo		-
68786-66-3	Triclabendazolo		-

RISULTATI: NUOVA SA?





POSITIVA

MEBENDAZOLO

FLUBENDAZOLO

POSITIVA

Genotoxicity of flubendazole and its metabolites *in vitro* and the impact of a new formulation on *in vivo* aneugenicity

David J. Tweats*, George E. Johnson, Ivan Scandale[1], James Whitwell[2] and Dean B. Evans[1]

2016

Il FLUBENDAZOLO in nuove formulazioni risulta **positivo** al test del MN in vivo!

Il sito di legame della Tubulina continua a non essere chiaro

AGGIORNAMENTO ISSMIC

Laboratory of Mathematical Chemistry
LMC oasis
In vivo Micronucleus Database

Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (Groups 1, 2A and 2B)
The summary report of the 6th collaborative study by CSGMT/JEMS · MMS

Takeshi Morita [a], Norihide Asano [b], Takumi Awogi [c], Yu F. Sasaki [d], Sei-ichi Sato [e], Hiroyasu Shimada [f], Sizuyo Sutou [g], Takayoshi Suzuki [h], Akihiro Wakata [i], Toshio Sofuni [h], Makoto Hayashi [h,*]

EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing
Genotoxicity and Carcinogenicity
Consolidated Database

DATO BIOLOGICO «GREZZO»

2B-62 β-Propiolactone [57-57-8] [Fig. 2B-62]

IARC Mono., 4, 259–269 (IARC, 1974a)

The 6th CSGMT MN assay (***Negative***) MMS Commun, 2, 109–116 (1994)

No micronucleus induction was observed in ddY mouse bone marrow or peripheral blood 24 h after double intraperitoneal treatments of up to the LD_{50} per treatment. A reduction of the polychromatic erythrocyte to total erythrocyte ratio was observed at the high dose group in the bone marrow test (data not shown).

Criteri di valutazione dei dati conformi a ISSMIC

TOXICITY BIOMARKER

	Chemical	MN assay result *	Tissue examined	Highest dose	Mortality at the highest dose	PCE (RET) ratio**	In vitro CA***
2B-59	Phenoxybenzamine HCl	–	BM	>LD_{50}	+	+	nd
2B-60	Ponceau 3R	–	PB	LD_{50}	+	+	nd
2B-62	β-Propiolactone	–	BM&PB	100	+	+	+
2B-64	*o*-Toluidine	–	BM	>LD_{50}	all	–	+
2B-67	Trypan blue	I	BM	40%LD_{50}(–)		–	–

13 SOSTANZE NEGATIVE + 26 SOSTANZE POSITIVE + 12 SOSTANZE BENZIMIDAZOLICHE = 51 SOSTANZE

CONCLUSIONI

Problema di natura informatica di Toxtree nell'interpretazione di alcune classi chimiche

Nuova definizione dell'allerta 32 sul piano meccanicistico

1,3 di-alcossibenzene → 1,3 di-metossibenzene

È stata identificata una nuova potenziale allerta

(lo *scaffold* benzimidazolico)

Sono state identificate 51 sostanze idonee per l'aggiornamento di ISSMIC


R3
R4
N
R2
R5
N
R6
R1
GRAZIE DELL'ATTENZIONE