Utilizzo di metodi *in silico* e *read-across* per la valutazione di miscele

Emilio Benfenati
Laboratory of environmental Chemistry and Toxicology

Workshop Valutazione degli effetti combinati delle miscele di sostanze chimiche
teleconferenza, 25·06·2020

ISTITUTO DI RICERCHE FARMACOLOGICHE MARIO NEGRI · IRCCS

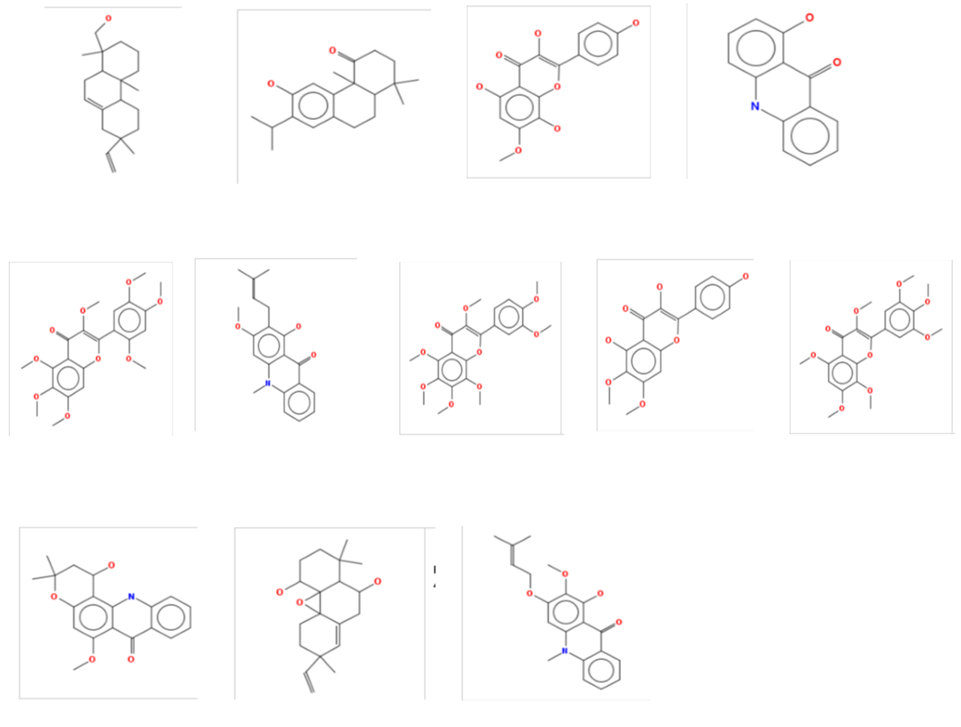
Possibili utilizzi dei metodiche in silico per le miscele: **effetto** o ***esposizione***

- **Predire informazioni su sostanze singole con dati mancanti**
- **Utilizzo per sostanze UVCB**
- **Raggruppamento sostanze comuni (ad esempio per mode of action, per applicazione strategie tipo dose/concentration addition)**
- **Predire effetti combinati**
- ***Predire diversi comportamenti ambientali***
- ***Modelli per tossicocinetica***

Computer come “cervello” o “muscolo”

Modelli in silico per completare dati mandanti su effetto

- Per predire molti endpoints, su molte sostanze, tempi rapidi:
- *Dafnia, alga, pesce, acuto/cronico, acqua dolce/salata, micro-organismi, terrestre, insetti, lombrico, uccelli, piccolo mammiferi*
- *Interferenti endocrini (diversi endpoints)*

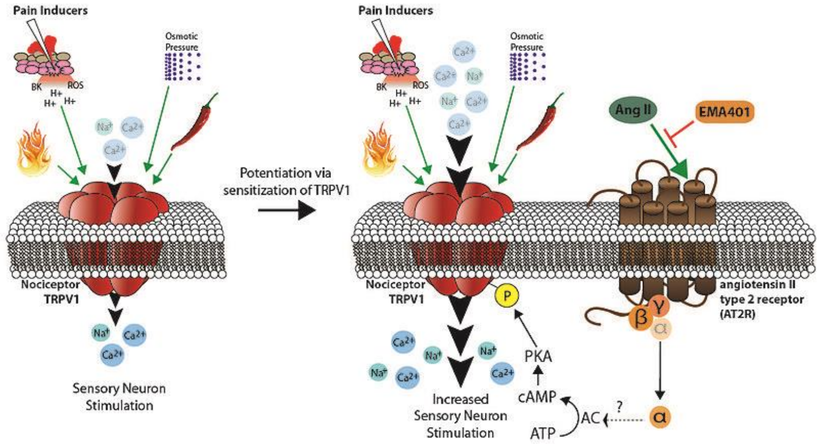


Utilizzo per sostanze UVCB: esempio dei surfattanti

- Sulfonati e analoghi
- Differente numero di atomi di carbonio
- Differente insaturazione
- Software può generare tutte le possibilità e calcolare effetti di tutti i possibili membri della famiglia: quindi analisi di trend, cluster, etc.

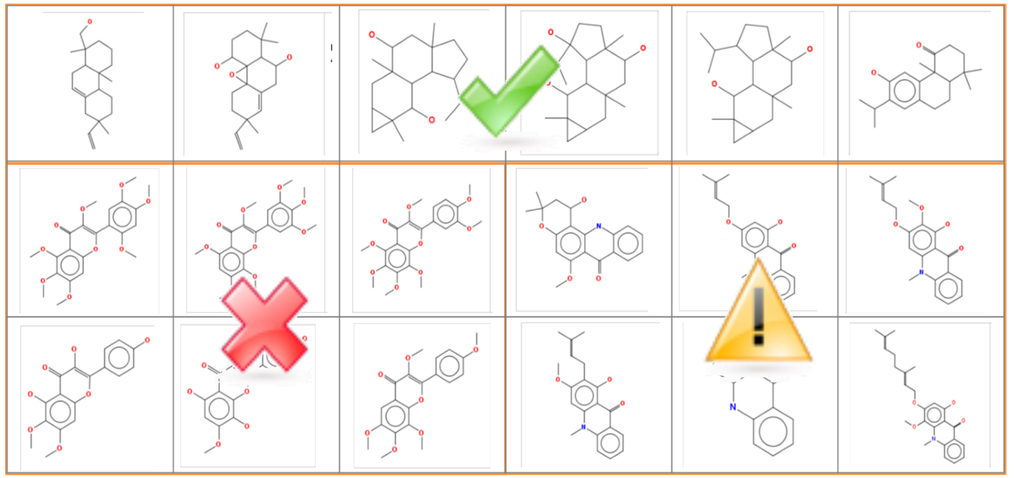
Modelli in silico per predire *mode of action* (MoA) per uso secondo *concentration addition* (CA)

- Pesce (dafnia): TEST, VEGA
- Interferenti endocrini
- Pesticidi
- Genotossicità
- (AOPs)



Raggruppamenti secondo cluster non pre-definiti

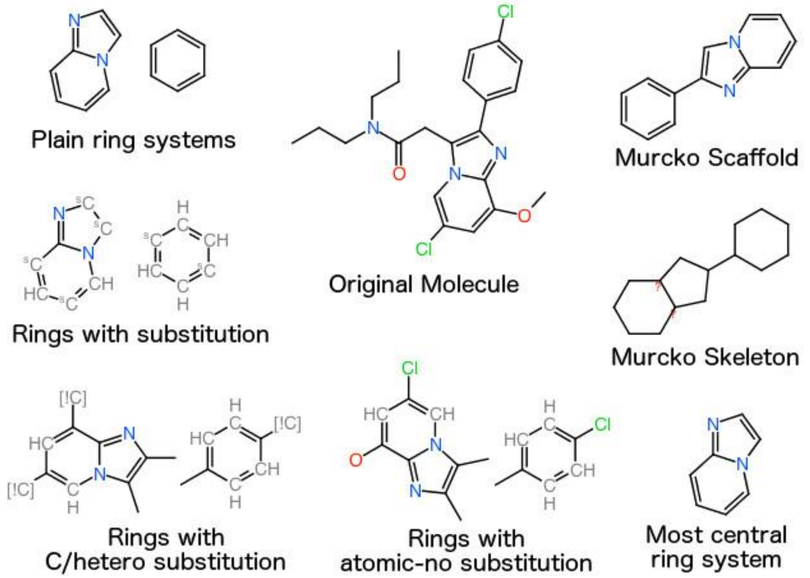
- Identificazione di famiglie
- Presenza di rappresentanti con dati sperimentali
- Verifica per le altre sostanze (read-across/grouping)



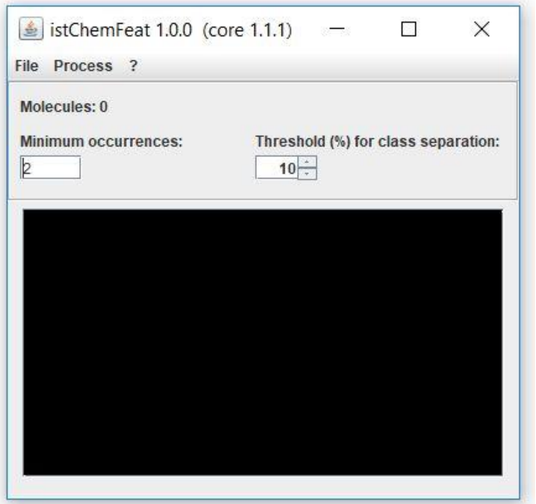
Unsupervised clustering

- Software per clustering
- Per costruire matrici, uno contro tutti

	Acid Black 1	Acid Black 24	Acid Blue 80	Acid Brown 235	Acid Brown 354	Acid orange 7	Acid Red 119	Acid Red 131	Direct Black 19	Direct red 23	Direct Red 81	Disperse Blue 79	Acid Yellow 42	Reactive Black 5	Reactive Red 195
Acid Black 1	1	0.803	0.741	0.819	0.841	0.769	0.808	0.859	0.772	0.848	0.767	0.772	0.768	0.842	0.763
Acid Black 2	1	0.803	0.741	0.819	0.841	0.769	0.808	0.859	0.772	0.848	0.767	0.772	0.768	0.842	0.763
Acid Black 24	0.803	1	0.802	0.815	0.844	0.733	0.868	0.831	0.742	0.852	0.781	0.692	0.779	0.773	0.752
Acid Black 26	0.845	0.916	0.789	0.853	0.887	0.742	0.892	0.864	0.755	0.869	0.781	0.718	0.8	0.787	0.772
Acid Blue 62	0.685	0.67	0.772	0.584	0.626	0.738	0.698	0.693	0.811	0.637	0.687	0.648	0.673	0.604	0.576
Acid Blue 80	0.741	0.802	1	0.712	0.761	0.67	0.791	0.779	0.861	0.746	0.771	0.676	0.766	0.706	0.687
Acid Blue 90	0.676	0.755	0.766	0.718	0.724	0.604	0.793	0.75	0.694	0.734	0.757	0.647	0.723	0.676	0.678
Acid Blue 113	0.821	0.964	0.804	0.817	0.84	0.748	0.902	0.848	0.758	0.845	0.795	0.708	0.795	0.77	0.749
Acid Blue 225	0.739	0.762	0.893	0.693	0.741	0.657	0.781	0.773	0.864	0.731	0.756	0.706	0.737	0.697	0.674
Acid Blue 260	0.748	0.783	0.91	0.695	0.74	0.689	0.804	0.787	0.865	0.732	0.773	0.675	0.743	0.692	0.666
Acid Blue 40 - 324	0.746	0.742	0.859	0.653	0.702	0.743	0.755	0.754	0.899	0.704	0.738	0.676	0.713	0.66	0.629
Acid Brown 58	0.77	0.776	0.67	0.94	0.865	0.581	0.734	0.747	0.648	0.817	0.674	0.648	0.712	0.825	0.845
Acid Brown 75/191	0.864	0.787	0.707	0.908	0.874	0.657	0.783	0.812	0.693	0.85	0.723	0.718	0.761	0.863	0.822
Acid Brown 191	0.838	0.8	0.709	0.883	0.87	0.671	0.784	0.79	0.692	0.833	0.726	0.717	0.76	0.848	0.811
Acid Brown 235	0.819	0.815	0.712	1	0.911	0.626	0.778	0.803	0.691	0.863	0.717	0.699	0.759	0.846	0.845
Acid Brown 348	0.874	0.8	0.726	0.901	0.906	0.676	0.802	0.82	0.714	0.863	0.745	0.725	0.776	0.846	0.804
Acid Brown 354	0.841	0.844	0.761	0.911	1	0.676	0.812	0.815	0.738	0.859	0.768	0.729	0.79	0.812	0.79
Acid Green 16	0.743	0.739	0.767	0.66	0.687	0.726	0.773	0.755	0.77	0.702	0.759	0.672	0.714	0.671	0.634
Acid Green 25	0.776	0.783	0.925	0.723	0.777	0.693	0.796	0.787	0.877	0.743	0.781	0.682	0.753	0.703	0.684
Acid Green 68	0.849	0.803	0.702	0.9	0.855	0.648	0.76	0.803	0.681	0.871	0.721	0.701	0.754	0.866	0.826
Acid Green 111	0.829	0.819	0.719	0.89	0.841	0.655	0.789	0.822	0.704	0.898	0.741	0.708	0.822	0.862	0.836



Software per Clusters per similarità strutturale o altro
istChemFeat / istCluster



“istChemFeat” extraction and classification of chemical features in a dataset

(available on https://chm.kode-solutions.net)

“istCluster”

Tools developed by Kode

istCluster: clustering based on the similarity (as calculated in VEGA)

	B	C
	CLUSTER	Mol ID
	*** Super-Cluster no. 1	
	* Cluster no. 1-1	
	O=C3C(=NNc1ccccc1)C(=Cc4cc(c(N=Nc2ccc(cc2)[N+](=O)[O-])c(N)c34)S(=O)(=O)O)S(=O)(=O)O	Acid Black 2 - 8005-03-6
	O=C(Nc3ccc4C(=O)C(=NNc2ccc(N=Nc1ccc(cc1)S(=O)(=O)O)cc2)C(=Cc4(c3))S(=O)(=O)O)c5ccccc5	Direct red 081 - 25188-42-5
	* Cluster no. 1-2	
	O=S(=O)(O)c6ccc(c(N=Nc4ccc(N=Nc3ccc(Nc1ccccc1)c2c3(cccc2S(=O)(=O)O))c5ccccc45)c6)Cl	Acid Blue 345 - 97889-95-7
	O=S(=O)(O)c6ccccc(N=Nc4ccc(N=Nc3ccc(Nc1ccccc1)c2c3(cccc2S(=O)(=O)O))c5ccccc45)c6	Acid Blu 113 - 3351-05-1
	O=S(=O)(O)c4cc(O)c5c(N=Nc3ccc(Nc1ccccc1)c2c3(cccc2S(=O)(=O)O))cc(cc5(c4))S(=O)(=O)O	Acid Blue 092 - 3861-73-2
	O=S(=O)(O)c6ccccc(N=Nc4ccc(N=Nc3ccc(Nc1ccccc1)c2c3(cccc2S(=O)(=O)O))c5ccccc45)c6	Acid Blue 113 - 3351-05-01
	O=S(=O)(O)c6ccccc(N=Nc4ccc(N=Nc3ccc(Nc1ccc(cc1)C)c2c3(cccc2S(=O)(=O)O))c5ccccc45)c6	Acid Blue 120 - 3529-01-9
	O=S(=O)(O)c5cccc(N=Nc3ccc(N=Nc1ccc(cc1)N(Cc2cccc(c2)S(=O)(=O)O)CC)c4ccccc34)c5	Acid Red 119-A - Acid Red 119-C - 70210-06-9
	O=S(=O)(O)c7cccc6c(N=Nc4ccc(N=Nc3ccc(Nc1ccccc1)c2c3(cccc2S(=O)(=O)O))c5ccccc45)cccc67	Acid Black 024-A - 3071-73-6
	O=S(=O)(O)c6ccc5c(ccc(O)c5(N=Nc3ccc(N=Nc2ccc(Nc1ccccc1)c(c2)S(=O)(=O)O)c4ccccc34))c6	Acid Black 026 - 6262-07-3

(currently only *in-house*)

Modelli in silico per predire LD50 di pesticidi in miscele

Input

ID	SMILES Chemical A	Mode of ActionA	TUa	SMILES Chemical B	Mode of ActionB	Tub	LD50	Additive(sm(ii)/an iii)
1	CC(=NC#N)N(C)CC1=CN=C(C=C1)Cl	Nicotinic acetylcholine receptor (nAChR) a	0.165	CCCCOCCCOCCOCC1=	P450-dependent monooxygenase inhibitor	0.030	1.17	ii
2	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	0.248	CCCCOCCOCCOCC1=	P450-dependent monooxygenase inhibitor	0.030	5.04	ii
3	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	1.034	CC(C)C(C(=O)OC(C#N	Sodium channel modulators_Nerve action	0.010	20.97	i
4	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	0.629	CC(C)C(C(=O)OC(C#N	Sodium channel modulators_Nerve action	0.030	12.77	ii
5	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.227	CCOP(=S)(OCC)OC1=	Acetylcholinesterase (AChE) inhibitor	0.150	1.53	ii
6	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.031	CCOP(=S)(OCC)OC1=	Acetylcholinesterase (AChE) inhibitor	0.490	0.21	ii
7	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.039	CCOP(=S)(OCC)OC1=	Acetylcholinesterase (AChE) inhibitor	0.150	0.78	ii
8	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.121	CC1=NN(C(=C1C=NO	Mitochondrial complex I_electron transport	0.060	2.4	ii
9	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.189	CC1=CC(=C(C=C1)N=	Octopamine receptor agonists	0.020	3.74	ii
10	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.515	CC1=CC(=C(C=C1)C(C	Phenol	0.181	10.2	ii
11	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.224	COC(=O)N(C1=CC=CC	Mitochondrial complex III_Quinone outside I	0.100	4.43	ii
12	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.586	C1=CC=C(C(=C1)C2=C	Complex II_Succinate-dehydrogenase inhibit	0.100	11.6	ii
13	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.366	C(#N)C1=C(C(=C(C(=C	Multi-site contact activity_unspecified mech	0.160	7.24	ii
14	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.001	CCCN(CCOC1=C(C=C(	P450-dependent monooxygenase inhibitor_I	0.070	0.01	ii
15	CC(C)C(C(=O)OC(C#N)C1=CC(=CC=C1	Sodium channel modulators_Nerve action	0.001	CCCCOCCOCCOCC1=	P450-dependent monooxygenase inhibitor	0.030	0.01	ii
16	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	0.208	CC(C)C(C(=O)OC(C#N	Sodium channel modulators_Nerve action	0.080	6.5	ii
17	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	0.132	CC1=NN(C(=C1C=NO	Mitochondrial complex I_electron transport	0.060	4.12	ii
18	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	0.295	CC1=CC(=C(C=C1)N=	Octopamine receptor agonists	0.020	9.2	ii
19	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	0.740	CC1=CC(=C(C=C1)C(C	Phenol	0.181	23.1	i
20	CCOP(=S)(OCC)OC1=CC2=C(C=C1)C(=	Acetylcholinesterase (AChE) inhibitor	0.724	C1=CC=C(C(=C1)C2=C	Complex II_Succinate-dehydrogenase inhibit	0.100	22.6	i

Modeling

$$DCW(T^*, N^*) = \sum_{k=1}^{NA} CW(S_k) + \sum_{k=1}^{NA-1} CW(SS_k) + \sum_{k=1}^{NA-2} CW(SSS_k)$$

Output

$R^2 \approx 0.80$ (across the four different splits)

Split	Set	n	R^2	CCC	Q2	RMSE	F
1	TRN	25	0.974	0.986	0.968	0.191	863
	iTRN	24	0.981	0.959	0.977	0.297	1140
	CLB	24	0.847	0.920	0.808	0.303	122
	VLD	24	0.888			0.527	
2	TRN	25	0.957	0.978	0.947	0.246	512
	iTRN	24	0.957	0.956	0.950	0.307	492
	CLB	24	0.785	0.883	0.726	0.346	81
	VLD	24	0.807			0.621	
3	TRN	24	0.959	0.979	0.951	0.206	522
	iTRN	24	0.954	0.948	0.943	0.333	462
	CLB	24	0.741	0.835	0.693	0.548	63
	VLD	25	0.750			0.605	
4	TRN	24	0.936	0.967	0.922	0.286	324
	iTRN	24	0.936	0.962	0.915	0.289	325
	CLB	24	0.858	0.919	0.8395	0.454	133
	VLD	25	0.777			0.514	

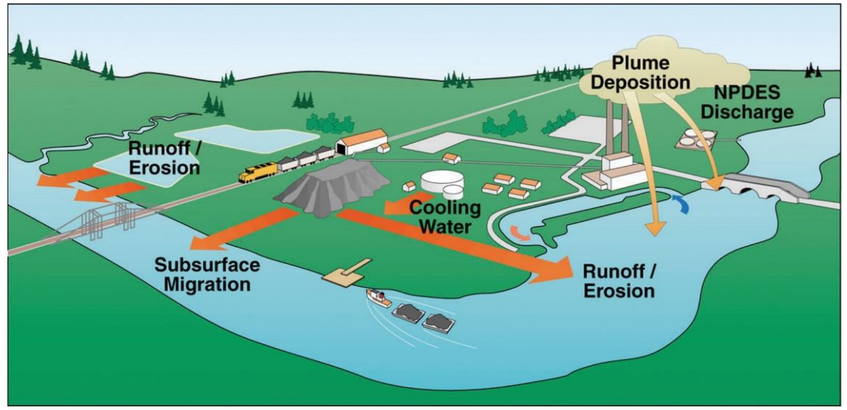
Modelli in silico per predire LD50 di pesticidi in miscele (2)

Carnesecchi E, Toropov AA, Toropova AP, Kramer N, Svendsen C, Dorne JL, Benfenati E
Predicting acute contact toxicity of organic binary mixtures in honey bees (A. mellifera) through innovative QSAR models
Science of the Total Environment, 2020, 704, 135302, https://doi.org/10.1016/j.scitotenv.2019.135302

Carnesecchi E, Svendsen C, Lasagni S, Grech A, Quignot N, Amzal B, Toma C, Tosi S, Rortais A, Cortinas-Abrahantes J, Capri E, Kramer N, Benfenati E, Spurgeon D, Guillot G, Dorne JLCM
Investigating combined toxicity of binary mixtures in bees: Meta-analysis of laboratory tests, modelling, mechanistic basis and implications for risk assessment
Environment International, 2019, 133, 105256, https://doi.org/10.1016/j.envint.2019.105256

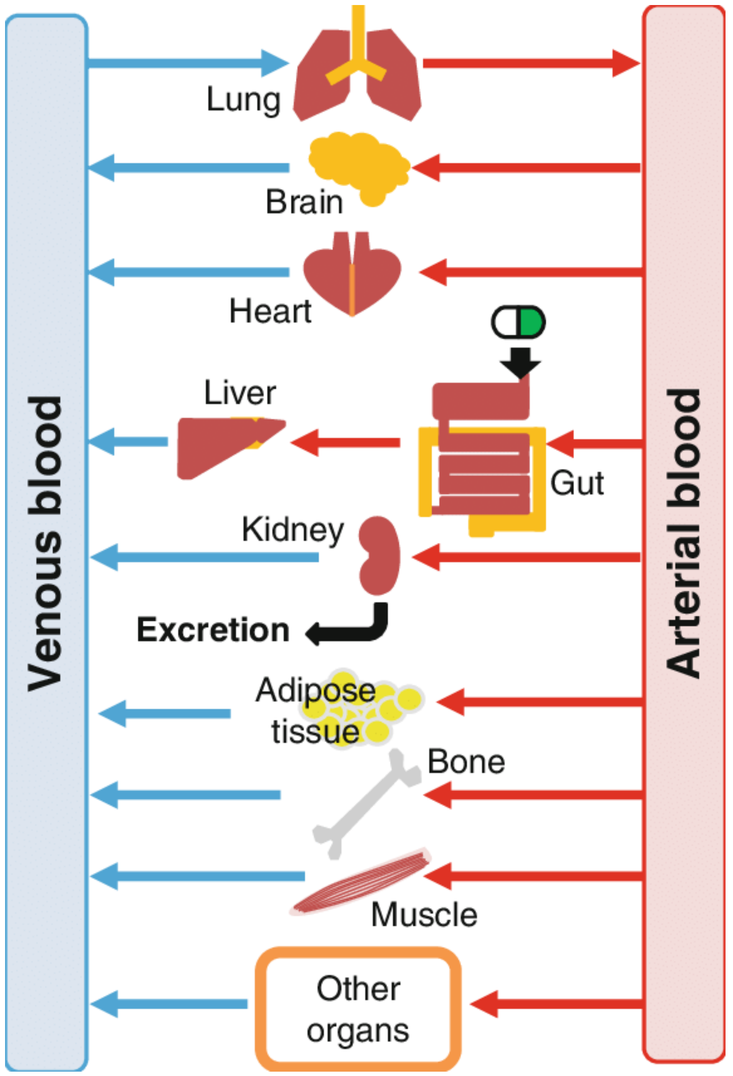
Modelli in silico per comportamenti ambientali. Variazione spazio-temporale della miscela

- Variazioni dovute a diverso comportamento ambientale
- Progetto VERMEER: Scenari disperdenti, contaminazione da petrolio, biocidi....
- Modelli SPHERA
- Differenze quantitative e qualitative fra miscele «esterne» ed «interne» (BCF)



Possibilità di modellazione di interazioni complesse. Tossicocinetica

- Substanza A influenza substanza B modificandone il metabolismo o altri processi (ADME)
- I modelli in silico possono simulare il metabolismo / ADME



Conclusioni

- Miscele: problema complesso, diversi tools in silico
- Sfide:
  - Disponibilità dei dati
  - Identificazione delle deviazioni da Concentration addition
  - Studi su aspetti biologici
  - Esposizione è solo a miscele

Grazie

ISTITUTO DI RICERCHE
FARMACOLOGICHE
MARIO NEGRI · IRCCS