Emilio Benfenati
Istituto Mario Negri

Ralf Knauf
Federchimica - Centro Reach S.r.l.

I Primi Risultati dei Progetti Europei ORCHESTRA e ANTARES sui Metodi Alternativi

6° Conferenza Sicurezza Prodotti: REACH • Federchimica & AssICC - Milano • 3 febbraio 2011

Alternative Non-Testing methods Assessed for REACH Substances

LIFE08
ENV/IT/000435

MARIO NEGRI
ISTITUTO DI RICERCHE FARMACOLOGICHE

ISTITUTO SUPERIORE DI SANITÀ

FEDERCHIMICA
CONFINDUSTRIA

POLITECNICO DI MILANO

www.antares-life.eu

REACH and QSAR

(Q)SAR is mentioned among
other alternatives to animal testing

AS AN *ACCEPTABLE METHOD* TO *FILL DATA GAP*

According to *REACH regulation* (Annex XI)
a (Q)SAR is VALID if:

the model is recognized scientifically valid;

the substance is included in the *applicability domain* of the model;

results are adequate for classification and labelling and for risk assessment;

adequate documentation of the methods provided.

Main Actions

Action 1 **Survey of current methods** for REACH

Action 2 Identification of the **criteria for the non-testing methods** for REACH

Action 3 **Identification of** suitable **experimental databases/data sets** for the ecotoxicological, toxicological and environmental endpoints for REACH

Action 4 **List of (Q)SAR models** for REACH, and their review

Action 5 **Validation** of non-testing methods (incl. read-across)

Action 6 Identification of **boundaries for best use** of models (applicability domain) and of the **assessment factors**

Action 7 **Architecture for integration** of non-testing methods

SURVEY OF CURRENT METHODS FOR THE COMPLIANCE TO THE REACH LEGISLATION (I)

STEP 1

•Launch of contacts to existing laboratory structures in Italy to involve at least 20 Italian laboratories.

•First screening information from those 20 companies about their locations, analytical and testing capabilities, and the type of certification declared by the laboratories.

•Inquiry with full Reach endpoints list with OECD guidelines was sent to and completed by the laboratories.

•Beside national laboratories also 4 important European laboratories had been contacted – only 1 gave a reply and concrete contribution.

SURVEY OF CURRENT METHODS FOR THE COMPLIANCE TO THE REACH LEGISLATION (II)

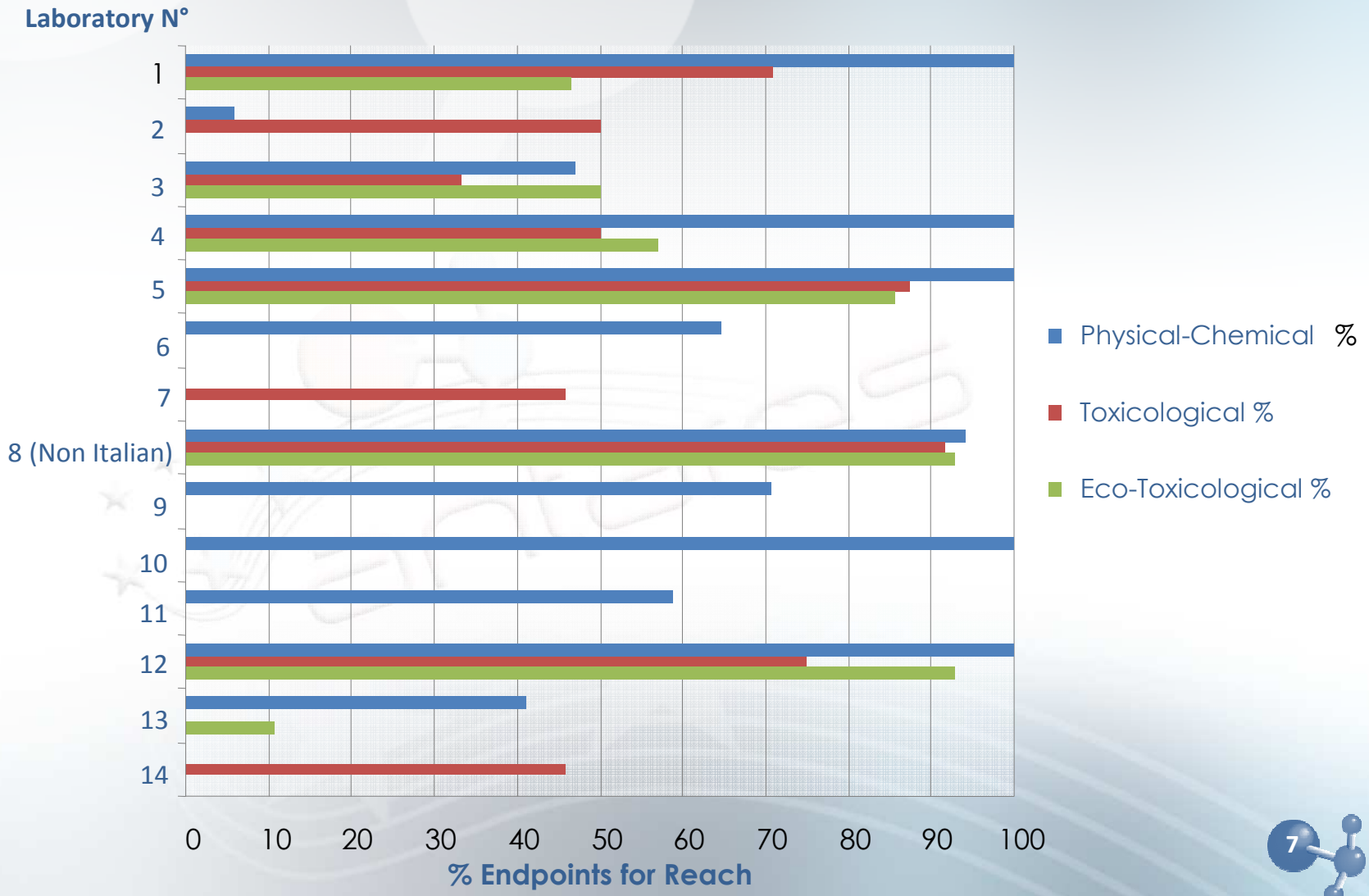
STEP 2 (i)

FEDERCHIMICA + CENTRO REACH evaluated all details of the survey coming from 13 national + 1 European laboratories:

- 10 national laboratories cooperated ➔ GLP Certification
- 3 national laboratories cooperated ➔ without GLP (some in progress)
- 1 European laboratory ➔ GLP Certification

Action 1

REACH ENDPOINT LABORATORY CAPABILITIES



SURVEY OF CURRENT METHODS FOR THE COMPLIANCE TO THE REACH LEGISLATION (II)

STEP 2 (ii) : OVERVIEW on TESTING CAPABILITIES

- 5 Italian companies offer tests min. 50 % of Reach tox endpoints.
- 4 can conduct min. 50 % of REACH ecotox endpoint testing.
- Some have capability for in vitro/alternative testing.
- European lab covers more than 90 % of all requirements.
- Best Italian laboratory has similar capabilities.

SURVEY OF CURRENT METHODS FOR THE COMPLIANCE TO THE REACH LEGISLATION (III)

NEXT STEPS

•Federchimica/Centro Reach will keep monitoring of the Italian laboratory situation for REACH testing.

•Complete evaluation and upgrading of labs with GLP certification for tox and ecotox testing activities necessary to run studies for REACH Regulation.

•Federchimica/Centro Reach will arrange interviews with several stakeholders.

•The REACH registration deadline 2013 will involve more substances which need new data/studies compared to the 2010 registrations where much data was/is already available.

•On 3.400 phase-in substances registered by 30 Nov 2010 for a total of more than 20.000 dossiers, only 500 dossiers had test-proposals (ca. 2,5 % !)

SURVEY OF CURRENT METHODS FOR THE COMPLIANCE TO THE REACH LEGISLATION (IV)

COST FOR REACH ENDPOINT TESTING

•Comparison of average costs of national laboratories with European references for same endpoint testing.

•These European references in general used for REACH registration activities in Europe.

•*Trend: average figures generally lower in Italy*.

•Less evident for long-term and very costly tests.

•Purely indicative since no lab is/was working for 2010 registration on such tests to our knowledge.

SURVEY OF CURRENT METHODS FOR THE COMPLIANCE TO THE REACH LEGISLATION (V)

NUMBER OF ANIMALS IN REACH TESTING

- Animal testing for the 1st registration deadline was extremely limited due to the official input to only submit testing proposals in case of missing data.

- Therefore Federchimica got only very few information about real executed tests with animals.

CRITERIA WITH SCORES FOR THE MODEL SELECTION

- Data and its quality
- Descriptors/fragments
- Algorithm
- Applicability domain
- Statistical results
- Validation
- Uncertainty/reproducibility
- Documentation

References: *ECHA documentation; OECD principles*

ENDPOINTS AND MODELS

PHYSICAL- CHEMICAL PROPERTIES	Log P, Solubility
ENVIRONMENTAL PROPERTIES	BCF, persistence
ECOTOXICOLOGICAL EFFECTS	Acute fish toxicity, acute daphnids toxicity
TOXICOLOGICAL EFFECTS	Skin sensitization, oral acute tox, carcinogenicity, mutagenicity

QSAR not only for registration as only source of info:

- For registration, also as support info *(weight of evidence)*;
- For CSA, also as support;
- For CLP;
- For prioritization of substances, for further check.

ORCHESTRA

Organising dissemination on Results of projects on CHemicals Evaluation, Spreading Techniques for Risk Assessment

www.orchestra-qsar.eu

Computational Models for Environmental Evaluation: A Case Study

9 EC Funded Projects considered as CASE STUDIES

Dissemination through a Web Portal: A RESOURCE FOR USERS

www.insilicomethods.eu

Development of Strategies for a Wider Dissemination and Exploitation

		NEEDS	BARRIERS	BENEFITS
REGULATORS Environmental protection, Chemical registration, Pharmaceuticals, Pesticides, Cosmetics, Biocides		• COVERAGE • REASONING • STANDARDIZATION • DEFINITION OF APPLICABILITY • TRANSPARENCY	• EXPERTISE NEEDED • RELIABILITY • LACK OF COMMUNICATION BETWEEN DEVELOPERS AND USERS	• IMPROVED RESPONSE TO REGULATORY REQUIREMENTS • REDUCTION OF ANIMAL TESTS • PRIORITIZATION
INDUSTRY Chemicals Pharmaceuticals Pesticides Food	REGULATORY PURPOSES	• SUPPORT • SUGGESTION • EASY-TO-USE • DEFINITION OF APPLICABILITY • REASONING • AUTOMATED REPORTING	• EXPERTISE NEEDED • REGULATORS' ACCEPTANCE • LACK OF TRANSPARENCY	• IMPROVED RESPONSE TO REGULATORY REQUIREMENTS • REDUCTION OF ANIMAL TESTS • PRIORITIZATION • HIGH-THROUGHPUT
	R&D	• SUGGESTION • CONFIDENTIALITY	• EXPERTISE NEEDED • METHODS NOT CONSIDERED POWERFUL • COSTS OF INITIAL SETTINGS	• REDUCTION OF COSTS/ANIMALS/TIME • HIGH-THROUGHPUT • ADDRESS SPECIFIC TARGETS/ENDPOINTS
SCIENTISTS Consultants on Information technology, Toxicology, Chemistry, Environmental sciences		• DEFINITION OF APPLICABILITY	• EXPERTISE NEEDED • REGULATORS' ACCEPTANCE • RELIABILITY	• REDUCTION OF COSTS/ANIMALS/TIME
NGOs Environmental protection Animal protection		• SAFETY • ANIMALS RIGHTS	• LACK OF EXPERTISE/KNOWLEDGE • LACK OF INFORMATION	• REDUCTION OF ANIMAL TESTS • HEALTH & ENVIRONMENT • SURVEILLANCE OF POLLUTANTS
CITIZENS Citizens associations Consumers associations		• SAFETY • ANIMALS RIGHTS	• LACK OF EDUCATION • LACK OF INFORMATION	• REDUCTION OF ANIMAL TESTS • HEALTH & ENVIRONMENT

OTHER ACTIVITIES

SWOT Analysis (Strength, Weakness, Threats, Opportunities) of EC projects OUTCOMES

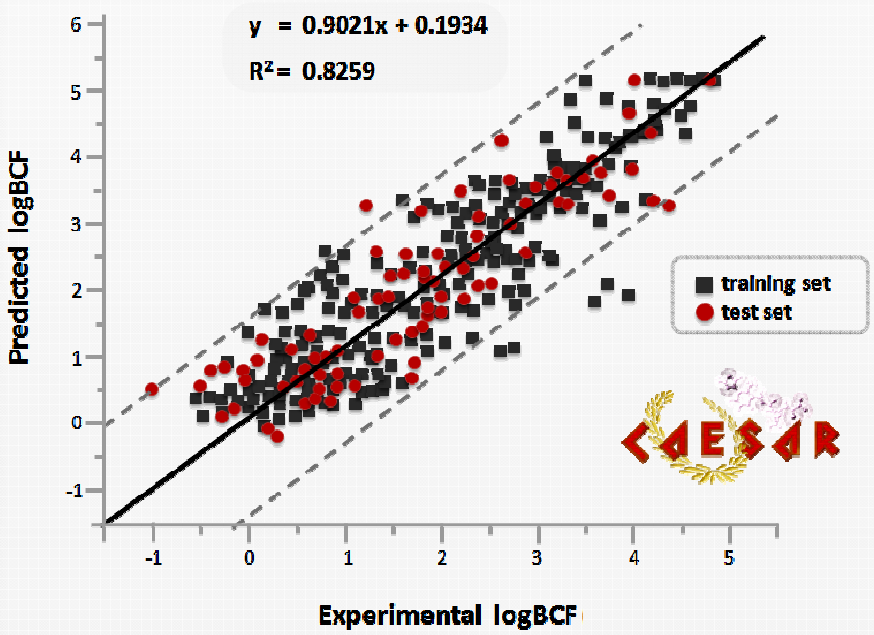
QUESTIONNAIRES to Stakeholders

WORKSHOP April 6, Milan
Discussion with regulators and stakeholders on the safe use of QSAR

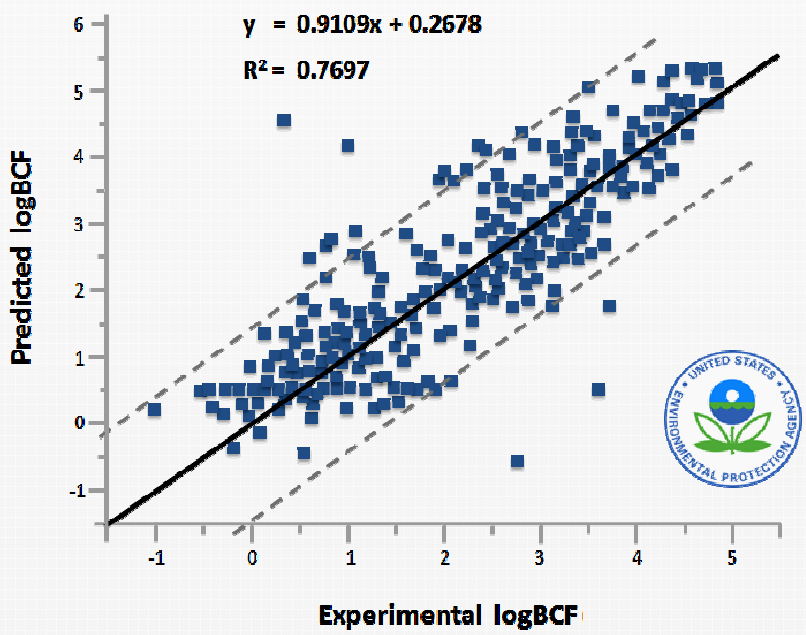
NEW PLATFORM FOR QSAR (WITH ANTARES)

CAESAR MODELLING FOR BCF

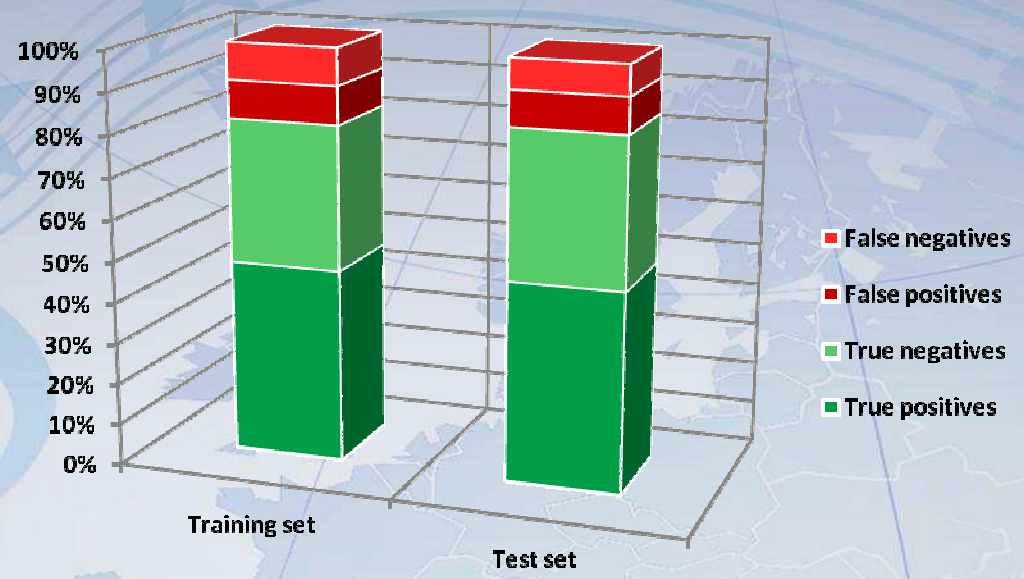
CAESAR MODEL



EPISUITE MODEL

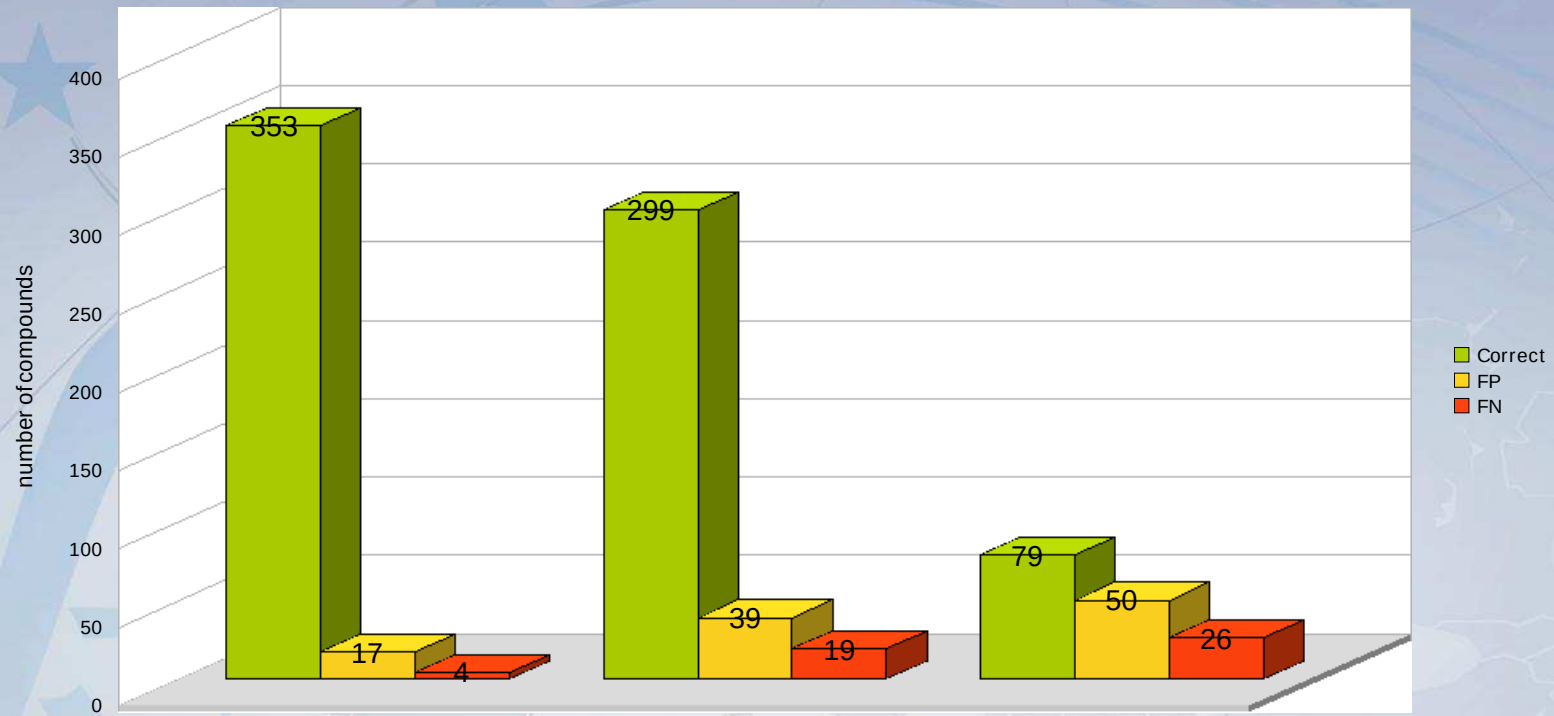


CAESAR MODELLING FOR MUTAGENICITY



- Good accuracy (considering reproducibility of the experimental data about 85%)
- A cost-sensitive model was also evaluated to reduce *FN*

Errors for Mutagenicity model on the Test-Set (836 compounds) for three Applicability Domain classes:



Prediction into AD

Prediction possibly out of AD

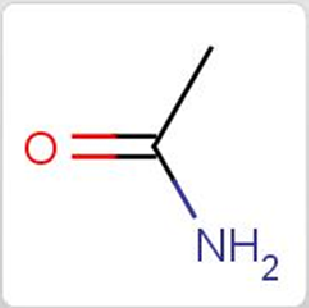
Prediction out of AD

THE APPLICABILITY DOMAIN TOOL

Developmental Toxicity

STEP 1 2 3 4

ID	IUPAC Name	SMILES	Assessment	
1	5-(butan-2-yl)-5-ethyl-1,3-...	CCC(C)C1(CC)C(=O)NC...	Developmental toxicant (compound into AD)	
2	acetamide	CC(N)=O	Developmental toxicant (compound possibly out of AD)	
3	(E)-N-ethylidenehydroxyl...	C\C=N\O	n.a. (compound out of AD)	
4	acetonitrile	CC#N	Developmental NON-toxicant (compound possibly out of AD)	
5	acetaldehyde	CC=O	Developmental NON-toxicant (compound into AD)	



MOLECULE ID	2
SMILES	CC(N)=O
IUPAC NAME	acetamide
PREDICTED VALUE	Developmental toxicant
APPLICABILITY DOMAIN	Predicted substance could be out of the Applicability Domain of the model.
ASSESSMENT	Developmental toxicant (compound possibly out of AD)

3

http://www.caesar-project.eu/

New Platform for QSAR

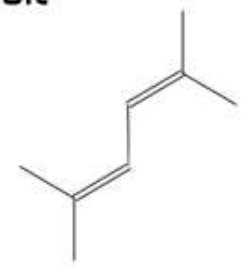
New Platform for QSAR

The Model Reasoning: MODEL A

FRAGMENTS | DESCRIPTORS

ID	IUPAC Name	Smile	Predicted Activity	Applicability Domain
3	(Z)-2,4,6-trimethylhept-3-ene	CC(C=C(CC(C)C)C)C	2.81	
413	1,3-benzothiazole-2(3H)-thione	SC1=Nc2c(cccc2)S1	0.82	
61	2,5-dimethyhexa-2,4-diene	C(C=C(C)C)=C(C)C	2.10	

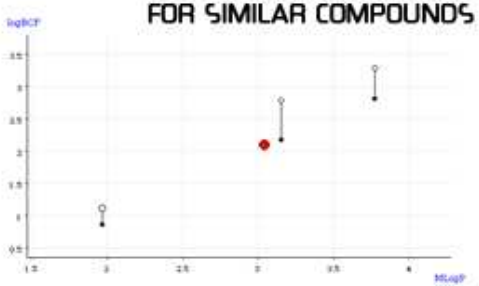
Molecule



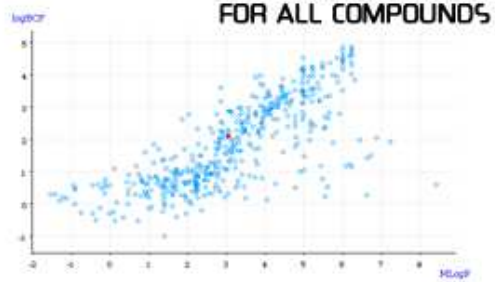
Comments

This result refers to LogP, the main descriptor

THE ROLE OF LOGP FOR SIMILAR COMPOUNDS



THE ROLE OF LOGP FOR ALL COMPOUNDS



Emilio Benfenati
Istituto Mario Negri

Ralf Knauf
Federchimica - Centro Reach S.r.l.

GRAZIE!