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NEWSLETTER

July 2011

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Evaluation of NTM and Laboratory Survey

Presentation of the Results

Alternative Non-Testing methods Assessed for REACH Substances

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LIFE08 ENV/IT/00435

ANTARES presented at Workshops and Conferences

6th Product Safety Conference: REACH

AssICC, Milan - February 11, 2011

The conference was organized by **FEDERCHIMICA** together with AssICC (Associazione Italiana Commercio Chimico). This event is a useful opportunity for discussion and comparison among the main stakeholders involved in REACH Regulation. Items like current state of implementation; latest news and views, unsolved issues and the difficult communication along the supply chain, registration dossier situation and testing tools have been discussed. ECHA, European Commission and CEFIC participated with speeches. More than 400 people participated to the event.

www.federchimica.it

Emilio Benfenati and Ralf Knauf gave a PRESENTATION during the afternoon session on **First results of European Projects ORCHESTRA and ANTARES on alternative methods**, and ANTARES Board and Project were presented.

ORCHESTRA Workshop

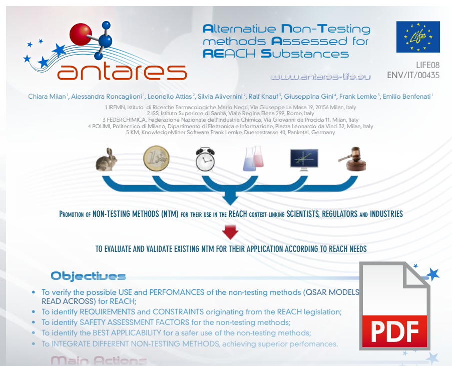
REACH & QSAR : What can we learn from Case Studies?

Mario Negri Institute, Milan - April 6, 2011

The **MARIO NEGRI INSTITUTE** organized the first WORKSHOP of the EC project **ORCHESTRA (Organising dissemination on Results of projects on Chemical Evaluation, Spreading Techniques for Risk Assessment)**, which involves also Politecnico di Milano and the Federchimica-related company Centro Reach S.r.l. The Workshop attracted 112 people from several European countries, 20 of which were regulators. Discussions were held on potentialities of QSAR methods for REACH and practical case studies.

www.orchestra-qsar.eu

ANTARES was presented through a POSTER with an **introduction to objectives and accomplished tasks.**



ANTARES presented at Workshops and Conferences

SETAC Europe 21st Annual Meeting

Milan, May 15-19, 2011

The **SETAC EUROPE 21ST ANNUAL MEETING** was held in Milan from 15-19 May, 2011. Chair of Local Organizing Committee was Emilio Benfenati, Mario Negri Institute, Coordinator of ANTARES.

SETAC, the **Society of Environmental Toxicology and Chemistry**, is a non-profit, worldwide professional society that deals with toxicology and environment.
At the conference participated more than 2,100 people from 67 different nations. The participants were not only researchers but also representatives of industry and governmental agencies. Dr Carlo Zaghi of Italian Ministry of Environment expressed satisfaction and success wishes for this important international event.

SETAC EUROPE
Milan 2011

http://milano.setac.eu

ANTARES was presented at SETAC with 2 POSTERS on project results about evaluation of non-testing methods and survey on laboratory structures (*see page 4-7*), 1 ORAL PRESENTATION and a COURSE on alternative methods.

Training Workshop on QSAR as a Tool in Chemical Risk Assessment

Lisbon, June 20, 2011

AP Tox – the **Portuguese Toxicology Association** – organized an ADVANCED TRAINING WORKSHOP with attention also to the basic concepts of QSAR modeling and to illustrate how QSAR can be used for chemical hazard identification. Speakers with extensive teaching and practical expertise in the various areas of QSAR modeling and regulatory toxicology gave their support.

http://regtox2011.webs.com/workshop1qsar.htm

Emilio Benfenati, among other expositions, gave a PRESENTATION during the afternoon session with the title **The ongoing EC projects ORCHESTRA and ANTARES.**

The SETAC 2011 POSTER on NTM Evaluation

The ANTARES project
An evaluation of non-testing methods for REACH

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5 KM, KnowledgeMiner Software Frank Lemke, Duererstrasse 40, Panketal, Germany

Objective

Promotion of non-testing methods (NTM) for their use in the REACH context linking scientists, regulators and industries

to EVALUATE and VALIDATE existing NON-TESTING METHODS (NTM) for their application according to REACH needs

The Identification of CRITERIA for Models Evaluation

ANTARES has the main target to analyze the use of NTM in accordance to REACH, and to identify suitable methods.

The study is organized as follows:

- to identify relevant criteria for comparing QSAR methods.
  A list of criteria has been established. They are divided into *main* (see *Table 1*) and *additional criteria.*
- to set a score for each criterion according to its importance.
- to identify available QSAR models which could be used for REACH.
- if available, to rank different models for the same endpoint, using the proposed *scoring system.*
- for equally scored models to evaluate also the additional criteria.

Table 1 : the main criteria for model validation

NAME	DESCRIPTION	SCORE
1. DATA QUALITY	To consider if the data set is the best quality for a specific endpoint.	0-3
2. NUMBER OF CHEMICALS	To consider the number of compounds.	0-3
3.DESCRIPTORS/FRAGMENTS	To give preference to models where explicit descriptors/fragments are defined.	0-3
4. EXPLICIT AND VERIFY THE ALGORITHM	To give preference to models where explicit algorithm is defined.	0-3
5. APPLICABILITY DOMAIN	Description of the AD of the model. Method used to assess the AD. Limits of AD.	0-3
6. PERFORMANCE	Statistical description.	0-3
7. VALIDATION	Internal and external validation.	0-3
8. OUTPUT	To explicit its format, to verify it and to test the comprehension for the user. To give preference to models where input is clearly defined, and how to use the results for REACH is explicated.	0-3
9. COST	To give preference to models which are less expensive.	0-3

The Model Availability

On the basis of the set of the identified criteria it is possible to analyze all endpoints. Not all models are fully representative of REACH endpoints. For instance, for reproductive toxicity models exist, which however do not completely fit with the characterization of the endpoint, as reported using classical methods.

ENDPOINTS	MODELS
7.1. State at 20°C and 101,3 kPa	-
7.2. Melting/freezing point	++
7.3. Boiling point	++
7.4. Relative density	+
7.5. Vapour pressure	++
7.6. Surface tension	+
7.7. Water solubility	++
7.8. Partition Coefficient n-octanol/water	++
7.9. Flash-point	+
7.10. Flammability	+
7.11. Explosive properties	-
7.12. Self-ignition temperature	-
7.13. Oxidising properties	-
7.14. Granulometry	-
7.15. Stability in organic solvents ..	-
7.16. Dissociation constant	++
7.17. Viscosity	+
8.1. Skin irritation or skin corrosion	
8.1.1. in-vivo skin irritation	+
8.2. Eye irritation	
8.2.1. in-vivo eye irritation	+
8.3. Skin sensitisation	++
8.4. Mutagenicity	
8.4.1. In-vitro gene mutation study in bacteria	++
8.4.2. In-vitro cytogenecity or micronucleus study in mamm. cells	-
8.4.3. In-vitro gene mutation study in mammalian cells	-
8.4.4. In-vivo mutagenicity study	+
8.5. Acute toxicity	
8.5.1. Acute toxicity - by oral route	++
8.5.2. Acute toxicity - by inhalation	+
8.5.3. Acute toxicity - by dermal route	-
8.6. Repeated dose toxicity	+
8.6.1. Short-term repeated dose toxicity (28 d)	+
8.6.2. Sub-chronic toxicity study (90 days)	+
8.6.3. A long-term repeated dose toxicity (> 12 m)	-
8.7. Reproductive toxicity	++
8.7.1. Screening for reproductive-/developmental toxicity	-
8.7.2. Pre-natal developmental toxicity study	-
8.7.3. Two-generation reproductive toxicity study	-
8.8. Toxicokinetics	++
8.8.1. Assessment of the toxicokinetic	-
8.9.1. Carcinogenicity study	++
9.1. Aquatic toxicity	
9.1.1. short-term toxicity testing on invertebrates (Daphnia)	++
9.1.2. Growth inhibition study aquatic plants (Algae preferred)	+
9.1.3. short-term toxicity testing on fish	++
9.1.4. Activated sludge respiration inhibition testing	+
9.1.5. Long-term toxicity testing on invertebrates (preferred Daphnia)	+
9.1.6. long-term toxicity testing on fish	+
9.1.6.1. Fish early-life stage (FELS) toxicity test	-
9.1.6.2. Fish short-term toxicity test on embryo and sac-fry stages	-
9.1.6.3. Fish, juvenile growth test	-
9.2. Degradation	
9.2.1. Biotic	
9.2.1.1. Ready biodegradability	++
9.2.1.2. Simulation testing on ultimate degradation in surface water	-
9.2.1.3. Soil simulation testing	-
9.2.1.4. Sediment simulation testing	-
9.2.2. Abiotic	
9.2.2.1. Hydrolysis as a function of pH	+
9.2.3. Identification of degradation products	+
9.3. Fate and behaviour in the environment	
9.3.1. Adsorption/desorption Screening	+
9.3.2. Bioaccumulation in aquatic species, preferably fish	++
9.3.3. Further information on adsorption/desorption	-
9.3.4. Info on the environ. fate and behaviour of the substance	-
9.4. Effects on terrestrial organisms	-
9.4.1. short-term toxicity to invertebrates	-
9.4.2. Effects on soil micro-organisms	-
9.4.3. short-term toxicity to plants	-
9.4.4. Long-term toxicity testing on invertebrates	-
9.4.6. Long-term toxicity testing on plants	-
9.5.1. Long-term toxicity to sediment organisms	-
9.6.1. Long-term or reproductive toxicity to birds	-

Table 2

- no suitable models about the specified endpoint;
+ some suitable models about the endpoint;
++ it is possible to find a certain number of models concerning the specified endpoint.

YELLOW: endpoints belonging to physical chemical group;
ORANGE: to toxicological properties;
BLUE: to ecotoxicological properties;
GREEN: to environmental properties.

Example of the Application of Criteria for Some Endpoint

ENDPOINTS	Data quality	number	descriptors	algorithm	AD	Performance	validation	output	cost
7.2. Melting/freezing point	3	0	3	3	0	3		3	3
7.3. Boiling point	3	2	3	3	0	3		3	3
8.3. Skin sensitisation	3	1	3	3	2	3	3	3	3
8.9.1. Carcinogenicity study	3	2	3	3	2	3	3	3	3
9.1.1. short-term toxicity testing on invertebrates (preferred species Daphnia)	3	1	3	2	2	2	2	3	3
9.1.3. short-term toxicity testing on fish	3	2	3	2	2	2	1	3	3
9.3.2. Bioaccumulation in aquatic species, preferably fish	3	2	3	3	3	2	2	3	3

Table 3 : Global overview of models characteristics for some selected endpoints

- On the basis of the criteria (*Table 1*), for a set of endpoints (from *Table 2*) we scored the models.
- This study will be applied to all endpoints to identify the most useful QSAR models.
- Then, we will evaluate read across on a set of selected cases, to evaluate also this approach.
- NTM will be validated using new chemicals.

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Alternative Non-Testing methods Assessed for REACH Substances

Grant Agreement LIFE08 ENV/IT/00435

Contribution for graphics: Vittorio Castiglioni, Istituto di Ricerche Farmacologiche Mario Negri

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An EVALUATION of NON-TESTING METHODS

ANTARES has the main target to analyze the use of NTM in accordance to REACH, and to identify suitable methods.

For this reason, the **criteria for the evaluation of alternative methods (QSAR and read across)** have been established considering the guidance document from ECHA, the general official guidelines, the requirements of the specific regulation (REACH), the RIP documents on REACH, the necessary quality of data, and the specific criteria for the algorithms.

A **survey of the criteria** for comparing QSAR methods has been done, assigning *scores for method evaluation*:

1. These criteria have been divided into main and additional criteria.
2. For each of them a particular score has been decided based on the importance of the criterion.
3. Using the identified criteria, ANTARES will identify available QSAR models which could be used for REACH (*not all models are fully representative of REACH endpoints*).
4. Then, if there is more than one method for the same endpoint, it will be possible to rank them, using the proposed scoring system.
5. For equally scored models, it will be possible to evaluate also the additional criteria.

The Main Criteria for Model Validation

NAME	DESCRIPTION	SCORE
1. DATA QUALITY	To consider if the data set is the best quality for a specific endpoint.	0-3
2. NUMBER OF CHEMICALS	To consider the number of compounds.	0-3
3.DESCRIPTORS/FRAGMENTS	To give preference to models where explicit descriptors/fragments are defined	0-3
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9. COST	To give preference to models which are less expensive.	0-3

Visit the ANTARES web portal for more details about the NTM Evaluation

The SETAC 2011 POSTER on Laboratory Survey

Alternative Non-Testing methods Assessed for REACH Substances

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LIFE08 ENV/IT/00435

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ANTARES is a **LIFE-funded project** (contract LIFE08 ENV/IT/0435), which stems from the need to verify the use and potential of alternative methods to animal testing for the REACH Regulation. For this it's necessary to understand the requirements and limits that the REACH legislation presents for alternative methods and identify factors and areas of best application for their safe use. The diffusion and the continuous improvement of these methods can not be separated from the integration and harmonization between them, as well as by an extensive and specific dissemination of their results.

Introduction, Objectives & Methods

The EU Regulation REACH, which entered into force in 2007 in the EU, represents today the most advanced system in the World to register, evaluate and authorize Chemical Substances.

According to latest estimates, about 40.000 substances will be processed in 3 phases, starting with high production volumes (from 1000 t) and the most concerning substances. The deadline of the 1st phase has been November 30, 2010.

To correctly evaluate the impact on the environment and human health, Industry will pay a high cost (billions of euros), and millions of animals might be sacrified to produce the necessary toxicity data.

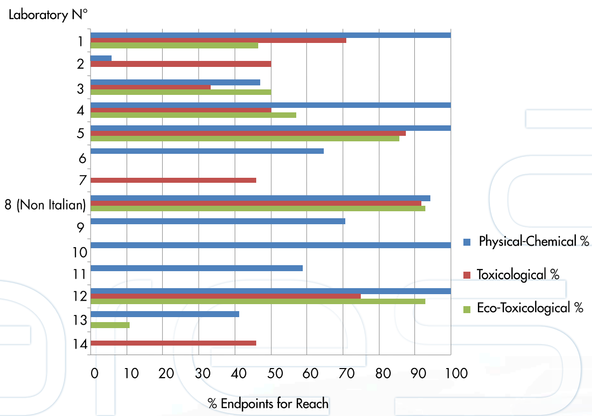
To limit these problems, REACH legislation promotes the use of non-testing methods, including Read Across and quantitative structure-activity relationship (QSAR). Read Across bases the evaluation of the unknown compound on the values of similar chemicals, while QSAR uses some chemical descriptors to evaluate the toxicity. Experience on the validity, predictivity and feasibility of these tools is still under discussion.

ANTARES WANTS TO EFFECTIVELY ADDRESS THE PROBLEM TO CHECK HOW TO USE THE NON-TESTING METHODS, FOR WHICH ENDPOINTS AND IN WHICH WAY

- ANTARES made an *overview* on current common methods to evaluate their practical availability, costs, number of animals, etc. to identify higher requirements for the alternative methods.
- Actions were taken to produce an *overview of laboratories which can provide the current tests.*

Results

REACH Endpoint Laboratory Capabilities in Italy



- Numerous contacts were launched with PUBLIC and PRIVATE LABORATORY STRUCTURES in ITALY.
- Most of the collaborating companies offer phys-chem testing, 7 laboratories offer toxicological and/or ecotoxicological testing.
- 12 Italian laboratories have indicated GLP certification.
- A well-known GLP certified European laboratory also collaborates in the project.
- 5 Italian companies offer tests for at least 50 % of REACH tox endpoints, 4 of them can conduct equal/more than 50 % of REACH ecotox endpoint testing.
- Some laboratory indicated in vitro/alternative testing capability.
- The non-Italian laboratory covers more than 90 % of all requirements, one Italian laboratory has same capabilities.

Conclusions

The monitoring of the Italian laboratory situation for REACH testing will continue to update the situation for GLP certification on tox and ecotox testing activities/capabilities.
The next registration deadline 2013 will involve many substances which need new studies compared to the 2010 registered substances.

The inventory here shown is not reflecting the complete picture of Italian laboratories, since it is based on information delivered by the companies

MARIO NEGRI ISTITUTO DI RICERCHE FARMACOLOGICHE — ISTITUTO SUPERIORE DI SANITÀ — FEDERCHIMICA CONFINDUSTRIA — POLITECNICO DI MILANO — KnowledgeMiner Software

Contribution for graphics: Vittorio Castiglioni, Istituto di Ricerche Farmacologiche Mario Negri

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A SURVEY of REACH ENDPOINT LABORATORY CAPABILITIES

ANTARES made an overview on current common methods to evaluate their practical availability, costs, number of animals, etc. to identify higher requirements for the alternative methods.

Actions were taken to produce an **OVERVIEW OF LABORATORIES WHICH CAN PROVIDE THE CURRENT TESTS**. A wide number of contacts to existing laboratory structures in Italy was arranged aiming to get at least 20 Italian laboratories involved in the the evaluation. We succeeded in obtaining *screening information from 20 companies* about their locations, analytical and testing capabilities, and the type of certification declared:

1. All laboratories are located in North and Central Italy. Most of these laboratories offer *phys-chem testing* and seven laboratories offer *toxicological and/or ecotoxicological testing*.
2. Not all companies have GLP (Good Laboratory Practice) certification, most have indicated SINAL certificate, the Italian system for test laboratories before GLP certification came into force.
3. FEDERCHIMICA made investigations with all these laboratories about *their capabilities for REACH endpoint testing* according to the official OECD guidelines.
4. FEDERCHIMICA contacted in parallel four well-known *European laboratories*, but only one replied to the questionnaire giving details helpful for this survey.

Finally 13 Italian + 1 European laboratories allowed to come to these conclusions:

- 5 Italian companies offer tests for at least 50% of REACH tox endpoints
- 4 of them can conduct equal/more than 50% of REACH ecotox endpoint testing.
- Some laboratory indicated in vitro/alternative testing capability.
- The non-Italian laboratory covers more than 90% of all requirements, one Italian laboratory has same capabilities.

The monitoring of the Italian laboratory situation for REACH testing will continue to complete it, in particular when companies will get GLP certification for tox and ecotox testing activities, which is the key to run studies under REACH Regulation.

The next registration deadline in 2013 will involve many substances which need new studies compared to the 2010 registered substances.

Visit the ANTARES web portal for more details

Cost for REACH endpoint testing

Difficulties incurred when inquiry came to COMMUNICATION OF COSTS FOR THE REACH ENDPOINT TESTS.
A comparison with average figures coming from other European surveys shows a general lower cost in Italy; this is less evident for long-term and very costly tests.

Info on Animals used for REACH

ANIMAL TESTING for the 1st registration deadline was extremely limited due to the official input to only submit testing proposals in case of missing vertebrate animal studies.
Therefore only very few information about real executed tests with animals is available to analyse the number of animals used for REACH testing.

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Evaluation of NTM
and Laboratory Survey
Presentation of
the Results

THE ANTARES NEWSLETTER PROVIDES REGULAR UPDATES AND INFORMATION ON NEWS FROM THE PROJECT.

The newsletter will be published every nine months, to highlight specific aspects and results on the project life and on the evaluation and assessment of alternative methods.

All numbers are published on-line at the ANTARES web portal.

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