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FINAL Report

Covering the project activities from 01/01/2010 to 31/12/2012

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LIFE+ PROJECT NAME or Acronym

ANTARES

Data Project

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1. List of content

2. Executive Summary

The main aim of the ANTARES (**A**lternative **N**on **T**esting methods **A**ssessed for **RE**ACH **S**ubstances) project was to reduce the gaps in knowledge concerning the Non Testing Methods (NTM), promoting them for their own use under the REACH legislation, linking scientists, regulators and industry in achieving this goal. ANTARES therefore has been intended to assess and validate existing NTM methods, in particular (Quantitative)Structure-Activity Relationship ((Q)SAR) models, in order to allow their application for regulatory purposes.

Specific expected objectives were:

1. to verify the possible use and performance of the existing NTM for REACH
2. to identify requirements and constraints originating from the REACH legislation about the use of NTM
3. to identify safety assessment factors for the NTM in order to increase their reliability
4. to identify the best applicability for a safer use of the NTM
5. to integrate different NTM achieving superior performance
6. to disseminate the results
7. to promote NTM for legislative purposes.

According to these objectives 7 technical actions (Actions 1 to 7) were planned in ANTARES plus 6 actions (Actions 8 to 13) related to non scientific aspects of the project:

ACTION 1. Survey of current methods for compliance to REACH legislation

ACTION 2. Identification of criteria for non-testing methods for REACH legislation

ACTION 3. Identification of suitable experimental databases/data sets for ecotoxicological, toxicological and environmental endpoints

ACTION 4. List of (Q)SAR models for ecotoxicological, toxicological and environmental endpoints for REACH, and their review

ACTION 5. Validation of non-testing methods

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

ACTION 7. Architecture for integration of different non-testing methods for best performances and coverage of applicability

ACTION 8. Communication and Dissemination

ACTION 9. Web Portal

ACTION 10. Project Management

ACTION 11. Monitoring

ACTION 12. Audit

ACTION 13. After-LIFE Communication plan

The **first Action** checked the existing structures in Italy capable to make the *experimental* tests requested in the REACH context. More than 20 Italian laboratories have been contacted and involved in this survey to obtain reliable information about testing capabilities. Overall, there is a lack of laboratories in particular for the (eco)toxicological endpoints. National authorities were interested on our survey.

The **second Action** identified the main criteria for the evaluation of the NTM, 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 was done, assigning scores for method evaluation. These criteria have been adopted within Action 4 to select a list of candidate models.

Action 3 gathered, commented and organised a series of databases offering useful data for a number of chemicals for the REACH relevant endpoints. These databases have been used to get the experimental data, to be used for the validation of NTM, within Action 4.

Action 4 listed existing models that can predict properties relevant for REACH. Considering the available, implemented software (freeware and commercial ones) and literature models for all REACH endpoints, 38 endpoints are covered with more than 250 software models; 70 of them are free. The list of software is on the ANTARES website, available to potential users (http://www.antares-life.eu/). This is itself a very valuable result of ANTARES, since such a list did not exist, and now is accessible through the project web site, with direct internet link to all these hundreds of models. We notice that further activities within ANTARES verified the performance of these models, which should be considered good only for the fact that we listed them. Action 4 identified the most suitable models to be assessed in Action 5. These are the selected endpoints:

Partition Coefficient n-Octanol/Water (substituted later by Solubility in Water)

Carcinogenicity Study

In-Vitro Gene Mutation Study In Bacteria

Acute Toxicity-By Oral Route

Short-Term Toxicity Testing On Invertebrates /Daphnia

Short-Term Toxicity Testing On Fish

Ready Biodegradability

Bioconcentration Factor.

In **Action 5** we evaluated the existing models. This was obtained by selecting appropriate sources of data, from the database identified in Action 2 to evaluate the best models identified in Action 4. This action was slightly delayed since in the literature appeared a similar work of validation for LogP so we changed this endpoint and selected solubility to be considered in action 5. All the analysis on the external validation have been completed on more than 50 models covering 8 endpoints (almost the double of the models originally expected to be covered) using large datasets of compounds. For each model we obtained an evaluation of suitability for its use on REACH and CLP requirements, supported by a large number of different statistical parameters (R^2, RMSE, accuracy, sensitivity, specificity, MCC etc.) in relation to the corresponding experimental values.

Action 6 was dedicated to characterise the boundaries when a certain NTM can be used or not. Indeed, for REACH a detailed information has to be provided if for a certain chemical a given NTM can be used. The behaviour of each model was evaluated in details by relating the errors of the prediction to the chemical identity of the wrong predictions, in order to identify possible general rules for errors useful to characterise the uncertainty of the prediction in relationship to certain areas of the method. In particular, the chemical classes which can be described by the model and the classes identifying the space of exclusion of the model were identified case by case. The analysis has been performed based on the response domains, i.e. a chemical has been included in the applicability domain of the model if it is not an outlier in terms of its response values.

Action 7 dealt with the definition of an architecture for the integration of different NTM to improve performance and wider the applicability of the models. We reviewed the integrating strategies proposed in literature and then we developed ours, taking into account the user needs caught in meetings with regulators. We worked on all endpoints with new strategies, which now are ready to use (e.g. BCF, mutagenicity), or at least with proposal for integration, even applying a software we had developed for our internal use to provide a new chunk of information (SarPy).

Action 8 had many good results on dissemination. ANTARES was mentioned in about 70 events, such as courses, conferences, workshops. We had meetings with European authorities, such as ECHA, EEA, EFSA, and Member States authorities. In particular Italian authorities were very interested in ANTARES and promoted an Italian network of laboratories on QSAR,

coordinated by the Coordinating Beneficiary, with Beneficiary ISS acting as Vice-coordinator, and in which Beneficiary POLIMI is also involved. Moreover, this action focused on the organization of the final workshop in Milan in November 2012.

Action 9 referred to the web site. The web site is http://www.antares-life.eu. It is active since February 2010 and it was updated on the average every week. It mentions the Life program, and it includes information on the project, and the beneficiaries. A series of pages provide free access to documents, such as deliverables of the project, the last of the events, a page with e-learning, containing material for beginners, but also an electronic book on QSAR and REACH, a set of resources, FAQs. There is also the list of the 250 QSAR models for REACH. A link sends to VEGA, the software which makes freely available a series of QSAR models.

Action 10 dealt with management. A person, Mrs Alessandra Roncaglioni, has been appointed and charged of this, working full time on ANTARES. We had four meetings, three in 2010 and one in 2011 (three in Milano and one in Rome). The mid-term review occurred in Milan, October 11 2011. A final review occurred in November 2012.

Action 11 dealt with monitoring. A Reviewers Committee has been appointed with representatives from Joint Research Center, and INERIS (France). Dr E. Mombelli (INERIS) participated at the final workshop in Milan on 21-22/11/2012 and sent an evaluation letter on 03/12/2012 with positive comments on the final results. Dr R. Corvi and Dr A. Worth (JRC) participated to the periodic meetings. Their preliminary and final comments (in a letter sent on 22/01/2013) were positive, too. Internally, Dr C. Milan, of the Coordinating Beneficiary, was responsible for the monitoring. Since she left the Mario Negri Institute in March 2012, Emilio Benfenati took the monitoring responsibility, with support by Mrs Azadi Golbamaki, from IRFMN.

The main output of ANTARES is the check of when a model can or cannot be used for 8 endpoints (>50 models). Reliable results can be expected for Water Solubility, Mutagenicity and BCF, while it is critical to evaluate other endpoints, such as Daphnia and LD50. Furthermore ANTARES provided a list of 250 NTM suitable for REACH, with 55 models tested for their validity using a specific evaluation protocol and comparing their outcomes with a huge number of experimental data retrieved from reliable databases.

Other important results come from the investigation on the stakeholders' needs and on the actual situation of laboratories involved in toxicological testing.

Moreover, ANTARES contributed to spread communications and information about in silico methods, on how to use them and when they are appropriate, with active presences in many workshops, seminars and conferences in different European countries.

Here there is a list of all the deliverables produced throughout the project :

Name of the Deliverable	**Code of the associated action**
1. Report on the survey of common methods for the compliance to the REACH legislation	1
2. Report on the identified criteria for non-testing methods, and their scores	2
3. List of end users	8
4. List of databases with assessment for REACH	3
5. Partnership agreement	10
6. Evaluation on the model performances for the first model (preliminary report)	4
7. Evaluation on the model availability for at least 20 endpoints (preliminary report)	4
8. First annual Management Report	10
9. First annual Monitoring Report	11
10. List and description of the dissemination activities	8
11. Production of info brochures, DVDs and newsletters	8
12. ANTARES Web portal	9
13. Evaluation on the model availability for all endpoints (preliminary report)	4
14. Results on the first 5 models, described in a preliminary report	5
15. Summary on the needs of stakeholders, barriers and initiatives to improve acceptability of non-testing methods	8
16. Evaluation on the model performances on at least 20 models (preliminary report)	4
17. Dissemination plan	8
18. Second annual Management Report	10
19. Second annual Monitoring Report	11
20. First report of the external Reviewers Committee	11
21. Report on the review of (Q)SAR models for REACH	4
22. Report on the protocol for the evaluation of non-testing methods	6
23. Results on the first 20 models, described in a preliminary report	5
24. Applicability domain for 20 models (preliminary report)	6
25. Report with results of the validation of non-testing methods for REACH	5
26. Report with the discussion and identification of the applicability domain for each validated model	6

27. Third annual Management Report	10
28. Third annual Monitoring Report	11
29. Report describing the architecture for the ideal integration of different non-testing methods	7
30. The After-LIFE communication plan	13
31. The Layman's report	8

3. Introduction

In order to ensure higher safety of chemical compounds and consequently a lower impact of these on human health and the environment, the European Union has introduced a new legislation for chemicals, REACH (Registration, Evaluation, Authorization and Restriction of Chemical substances - Regulation (EC) No.1907/2006 in force since June 1, 2007); it requires that all chemicals produced and/or placed on the EU market in quantities greater than or equal to 1 ton/year have appropriate safety information; in fact for each substance the industry is obliged to prepare a dossier containing information on physico-chemical, toxicological and ecotoxicological properties, to be sent to the European Chemicals Agency (ECHA) in order to obtain the registration and the subsequent authorization to manufacture and/or import.

REACH has therefore induced an urgent demand of toxicity tests for a large number of chemicals; the initial estimates talked about 100,000 registered substances for REACH by 2018, the year of the last deadline given by the regulation.

To meet these requirements, REACH provides that alternative methods to the use of animals (the so-called *in vivo* methods) shall be used, including Non-Testing Methods (NTM), able to predict the effects of the substance without its direct use but only on the basis of its structure (Article 13 of REACH). Indeed REACH, in addition to provide an increase in the level of protection of human health and the environment against the risks related to chemicals, has among its main objectives the promotion of alternative methods for the assessment of hazards deriving from chemical substances (Art.1 of REACH).

NTM comprise a range of instruments devoted to the estimation of physico-chemical, toxicological and ecotoxicological properties of chemical compounds based on the evaluation of the chemical structure. They include:

- structure-activity relationships, where a mathematical model is used to relate the chemical structure or the presence of some fragments with the investigated effect in a qualitative (SAR) or quantitative way (QSAR);
- expert systems, based on the codification into rules of human knowledge;

- read-across, based on the evaluation of existing experimental data for similar compounds that can be placed in the same category of chemicals.

Before carrying out experiments on animals, to be considered as a last resource of information on the toxicity of chemicals (REACH Art.25), the chemical industry should verify if alternative methods exist; however, today gaps still remain in the knowledge about which methods are available and can be used in practice.

Linking scientists, regulators and industries, ANTARES promoted the use on NTM in the context of REACH legislation by the assessment of available models for REACH relevant endpoints, testing them for new chemicals and defining their proper use in terms of boundaries for validity and proper integration of different methods to improve the performance.

4. Technical part

4.1. Task by task - description

4.1.1. Action 1 (Deliverable 1)

Survey of current methods for the compliance to the REACH legislation

Beneficiary responsible: FEDERCHIMICA

The first action served to provide a good starting basis relatively to the situation of the existing structures in Italy which may offer the possibility to make the experimental tests requested in the REACH context. Thus, this refers to the status of the availability of laboratories capable to deals with the REACH requirements, and not on the alternative non-testing methods, which is the content of the project. The activities done within this Action have been quite detailed, because the evaluation has been done not only on a formal point of view, on the theoretical possibility that a certain laboratory could make a certain test. For REACH a certain list of data has to be provided, and we followed that list. In addition, the procedure to get the value requires that data are obtained according to official protocols, and thus we checked that the laboratory was working according to the correct official scheme. Thus, a certain number of laboratories have to be excluded. In most of the cases public laboratories do not work according to the so-called “good laboratory practice” (GLP). This Action additionally checked the quality of the structure of the laboratory, with personal visits

and interviews. This provided an added value of this Action, which provided a realistic and detailed overview of the situation in Italy.

More than 20 Italian laboratories have been contacted and involved in this survey to obtain reliable information about testing capabilities quality-wise (if GLP certified or any other accreditation form was available) and about the number of REACH endpoint tests offered. 13 laboratories agreed to deliver detailed answer about testing capability on REACH endpoints.

Most of the laboratories offer phys-chem testing, 5 are able to conduct equal/more than 50% of toxicological endpoints testing, 3 more than 50% of ecotoxicological endpoints testing. The non-Italian laboratory included in our survey covers more than 90% of all requirements, and the best Italian laboratory has a similar capability.

Difficulties incurred when inquiry came to communication of costs for the tests.

Beneficiary Federchimica decided to communicate only average figures or a range since comparison of prices between competing parties are not in the scope of the action. Beneficiary Federchimica took advantage of its international relations and has also a list of average costs for same endpoint testing from German VCI. This list is very often used as reference in discussions for REACH registration activities in Europe. A comparison between both lists with average figures shows a general lower cost in Italy than for the German quotations; this is less evident for long-term and very costly tests. Those are purely indicative since no laboratory was working for 2010 registration on such tests to our knowledge. Animal testing for the first registration deadline was extremely limited due to the official input to only submit testing proposals in case of missing studies above those of 28 days toxicity studies.

The national authorities were interested in these results, including the Ministero dell'Ambiente and Ministero della Salute, and we provided the list of the laboratories. This also helped the national authorities to complete the overview of the available laboratories, and to identify needs. It was also an indicator of the many benefits brought by this action to the public community.

Briefly, there is a shortage of laboratory offering tests, in particular for toxicity endpoints, but also for ecotoxicological endpoints. A better situation exists for physico-chemical endpoints.

This Action was also reported at the SETAC international conference in Milan, May 2011, where we presented a poster. This Action was completed with a slight delay and the associated deliverable (D1) was later on improved according to the comments received by the Commission after the submission of the Inception Report.

4.1.2. Action 2 (Deliverable 2)

Identification of the criteria for the non-testing methods for the REACH legislation

Beneficiary responsible : IRFMN

The second action identified the main criteria for the evaluation of the non-testing methods. On the basis of the REACH text (in particular Annex XI) and the ECHA guidance and practical guides, the most important criteria for the acceptability of the QSAR models have been identified based on REACH. Unfortunately, the text of the regulation is quite short and no clue is given on how to address these items. For this, the guidance documents provided more details. The points listed within the QSAR Model Reporting Format from JRC and OECD principles for QSAR validation gave also explanatory reference to detailed points. Some were not fully relevant for us, while others needed some additional specification, and this has been done. The reason for the differences is that the OECD guidance structure is quite general, referring not necessarily to REACH, while within ANTARES we dealt with REACH. The main output of this Action, reported in D2, refers to the rationalization of the points addressed in these different documents to extract the most important criteria to be used in ANTARES for the evaluation of NTM and to set the scores associated to each criterion to be used in the evaluation of the available QSAR models in Action 4. This Action was also reported at the SETAC international conference in Milan, May 2011, where we presented a poster.

This Action was concluded in time, but after our visit to ECHA we further analyzed some aspects raised during our visit, hence the deliverable was updated later on.

4.1.3. Action 3 (Deliverable 4)

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

Beneficiary responsible: ISS

Retrieving data is crucial to both scientific and regulatory work. Among the sources of freely available data on chemical substances, one of the principal resources is the TOXNET database of the National library of Medicine. TOXNET is a cluster of different databases, collecting information on toxicology, hazardous chemical, environmental health, and toxic releases. From the web site, it is possible to search across and within the databases by several identifiers, such as Chemical Name, CAS Registry Number, Molecular Formula, Classification Code, Locator Code, and Structure or Substructure. Among the TOXNET

databases, the Chemical Carcinogenesis Research Information System (CCRIS) and GENE-TOX databases deal specifically with mutagenicity and carcinogenicity data. For example CCRIS contains over 8000 chemical records with animal carcinogenicity and mutagenicity test results. Test results have been reviewed by expert and all records are written in a standardized format. These databases typically utilize chemical names and CAS numbers which are non-unique and commercially registered and, therefore, unsuitable for a unique, public identifier. In addition, often the organization of the data follows that of the literature on paper, and does not lend easily itself to informatics implementation. Recently, concepts and computer techniques that originated from the structure-activity relationships science have provided powerful tools to create new types of databases, where the ability to retrieve data is strongly improved both in qualitative and quantitative terms. In fact, whereas the indexing elements in traditional databases, such as names and CAS number, are non-unique, prone to errors and devoid of intrinsic information, chemical structure as a chemical identifier has universally understood meaning and scientific relevance. Effective linkage of chemical toxicity data with chemical structure information can facilitate and greatly enhance data gathering and hypothesis generation in conjunction with (Q)SAR modelling efforts. Thus, a crucial point is that of collecting and standardizing portions of the existent knowledge in a way that allows: a) exploration across both chemical and biological domains; and b) structure-searchability through the data. These characteristics may be gained when chemical structures and toxicity data are incorporate into what is termed a Chemical Relational Database (CRD). CRD is a special type of relational database whose main informational unit is a chemical structure and whose fields are attributes or data associated with that chemical structure. In order to be accessed with a CRD application, the information has to be stored in specialized file formats. Among them, Structure Data File (SDF) format has become as the most widely used public standard for exchange of structure/data information on chemicals. SDF files are simple text files adhere to a strict format for representing multiple chemical structure records and associated data fields. An example of project designed to provide the user with self-contained data files that can be readily incorporated into CRD and used freely is the Distributed Structure-Searchable Toxicity (DSSTox) Database Network, which is a project of the USEPA. DSSTox efforts include the careful quality annotation and documentation of toxicity data in collaboration with toxicity data experts, and open public access to toxicity databases. At present, the DSSTOx data file cluster includes fourteen separate databases. Another database on chemical carcinogens is ISSCAN: “Chemical carcinogens: structures and experimental data”. The ISSCAN database contains information on chemical compounds

(more than 1000) tested with the long-term carcinogenicity bioassay on rodents. The ISSCAN initiative is aimed at providing the scientific and regulatory community with carcinogenicity calls that have been re-checked, in order to ensure the quality of the data. The data were cross-checked on different sources information available; contradiction were solved going back to the original papers, and results based on insufficient protocols were not included. Moreover the biological data were coded in numerical terms that can be used directly for QSAR analyses. This aspect of being QSAR-ready eliminates the intermediate passage of data transformation that often is problematic for the QSAR practitioner without specific toxicological expertise. These are the criteria for the identification of suitable experimental databases/data sets for the ecotoxicological, toxicological and environmental endpoints for REACH:

	Example of database: CCRIS
Database identification	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS
Endpoint identification	Carcinogenicity, mutagenicity, tumor promotion, and tumor inhibition
Data source characterisation	National Cancer Institute (NCI)
Quality check on the value	Test results have been reviewed by experts in carcinogenesis and mutagenesis
Presence of multiple/single values, uncertainty	Multiple values/uncertainty not resolved
Availability on information on details of values	Fully referenced data
Number of compounds	9,000
Type of input required for the query	Combination of words, chemical names, and numbers, including CAS Numbers.
Export capabilities	Text format download
Type of query: single or batch	Both
Dissemination level	Public

In conclusion, from the analysis of the 25 databases of this study, it can be deducted that:

1. There are a considerable number of carcinogenicity and mutagenicity data, however, ecotoxicity data are still inadequate.
2. The use of the most of databases, for QSAR model, requires the intermediate passage of data transformation that often is problematic without toxicological expertise.
3. Among the databases format, SDF is the QSAR-ready format; the ISSCAN, DssTox, Leadscope SAR Carcinogenicity and Leadscope SAR Genetox Databases present this format, however only DSSTox and ISSCAN databases are free.

With this input, beneficiary IRFMN selected the compounds to create the datasets for the evaluation of models of each endpoint selected, with a total number of more than 22000 values extracted. Deliverable 4 (D4) contains all the outputs expected for this Action, including the list of databases checked and the comparative tables. This information is publicly available through the ANTARES website. We did not encounter any particular problems during the work. The main problem concerns the lack of homogeneity of the various databases available on the web. Therefore in some cases it was difficult to categorize the various information contained in the databases. However Action 3 has been completed on schedule.

4.1.4. Action 4 (Deliverable 6, 7, 13, 16 and 21)

List of (Q)SAR models for the ecotoxicological, toxicological and environmental endpoints for REACH, and their review.

Beneficiary responsible: KM

The aim of action 4 was to identity the most suitable models to be assessed in Action 5. Therefore two main activities were included in this action. A first activity was to scrutinize existing models that can predict properties relevant for REACH. The final outcome of this activity (beside the preliminary report D7) was the full list of available software (freeware and commercial ones) and literature models for all REACH endpoints reported in D13. 38 endpoints were covered with more than 250 software models; 70 of them are free. The list of software is on the ANTARES website, available to all users (http://www.antares-life.eu/). We presented a poster at the SETAC international conference in Berlin, May 2012, with the results of this Action. We considered the endpoints present within REACH. For several endpoints there are many models available. We split these endpoints into four areas: Physico-chemical, environmental, ecotoxicological and toxicological groups.

From this overview of the available models, there are several models for physico-chemical properties, like partition coefficient and solubility. Furthermore, these models are in many cases based on a quite large number of experimental data. The occurrence of a large data set and of QSAR models is linked, since the availability of data is a fundamental need, and most models are built starting from collections of data available. The partition coefficient and the solubility are two physico-chemical properties which are also used to address other endpoints. For instance, most of the aquatic toxicity QSAR models use partition coefficient. This physico-chemical property is also used for fate models, like bioconcentration.

Among the fate endpoints, there are many models for bioconcentration. This is also related to the occurrence of hundreds of experimental values, and historically many models have been developed using logP, which is a classical descriptor within traditional QSAR models. Moreover a large number of animals (primarily fish) are necessary for performing this test.

Biodegradation is another endpoint for which there are many hundreds of experimental data.

Both bioconcentration and biodegradation are very important properties for REACH, because these properties have to be addressed to classify the chemicals as persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB).

In the set of endpoints for the ecotoxicological areas, acute fish toxicity and acute daphnids toxicity showed a quite large number of models. The reason for this is that, once again, there are many hundreds of data for these endpoints, and there is a tradition of modeling aquatic toxicity, mainly based on logP as a key descriptor.

For the human toxicity area mutagenicity has the largest number of data available, for several thousands of chemicals. Thus, also for this reason, many models have been developed for this endpoint. We have to remember that these data refer to the Ames test (an *in vitro* test on bacteria), while the number of data for the other mutagenicity studies is much more limited. There are also several models for carcinogenicity, which in many cases adopt schemes, like the check of toxic fragments, which are in common to the mutagenicity studies. In this case the number of experimental data is lower, as expected, but still a quite large one. For oral acute toxicity there are also many experimental data, and many models.

D6 and D16 focused on the selection of the most promising models among the full list of existing ones to pass them to Action 5 for their further assessment. D6 analyzed some statistical parameters to identify promising models while D16 on the basis of D6 and the criteria reported in Action 2 ranked these models. Considering existing models, their desirable characteristics and coverage of different types of endpoint for REACH the following endpoint were selected:

- 7.7 Water Solubility (phys-chem)
- 8.9.1 Carcinogenicity Study (human tox)
- 8.4.1 In-Vitro Gene Mutation Study In Bacteria (human tox)
- 8.5.1. Acute Toxicity-By Oral Route (human tox)
- 9.1.1 Short-Term Toxicity Testing On Invertebrates /Daphnia (ecotox)
- 9.1.3. Short-Term Toxicity Testing On Fish (ecotox)
- 9.2.1.1. Ready Biodegradability (environ. behavior)
- 9.3.2. Bioconcentration Factor (BCF) (environ. behavior)

About 50 QSAR models have been critically evaluated, to transmit the list to Action 5.

We notice that this figure, 50 models, is almost the double of what we promised within the contract. During our work we found a great expectation from stakeholders, indicating the usefulness of our results, thus we preferred to increase our work, to provide an even better overview.

Furthermore, as we mentioned, the initial physical-chemical endpoint, logP, has been substituted by water solubility, because we found a review with good results of this property already published. This caused to us some additional work, because we had already identified the datasets for logP and made some work on it. Anyhow, this resulted in the addition of some logP models in the VEGA website, thus it achieved anyhow some good results.

4.1.5. Action 5 (Deliverable 14, 23 and 25)

Validation of NTM

Beneficiary responsible: IRFMN

In action 5 the independent evaluation of existing models was foreseen. This was obtained by selecting appropriate sources of data, from the database identified in Action 2 to evaluate the best models identified in Action 4. For the 8 selected endpoint the most promising software were tested to verify their ability to estimate the property under evaluation. In several cases both freely available tools and commercial software were used.

In particular, we assessed the performance of 5 models for Water Solubility, 5 models for Ready Biodegradability, 9 models for Bioconcentration Factor, 8 models for Short-Term Toxicity Toxicity on Fish, 8 models for Short-Term Toxicity on Invertebrates/Daphnia, 7 models for Carcinogenicity, 8 models for In-vitro Gene Mutation and 5 models for Acute Toxicity-By Oral Route. So, the total number of QSAR models evaluated is 55, much higher than the 30 models initially planned (25 + 5 CAESAR models).

The data were analyzed according to different metrics to better reflect the characteristics of the different endpoints. In fact different statistical parameters were used for the evaluation of continuous endpoint (Water Solubility, Acute Toxicity-By Oral Route, Short-Term Toxicity Testing On Invertebrates /Daphnia, Short-Term Toxicity Testing On Fish and Bioconcentration Factor) and the categorical ones (Carcinogenicity Study, In-Vitro Gene Mutation Study In Bacteria and Ready Biodegradability). Here again the parameters calculated were more than what expected. For continuous endpoint, for example, the evaluation was also done in a categorical manner to evaluate the performance for CLP thresholds, in addition to R^2, intercept, RMSE and Maximum error. For some endpoint, where the data were not balanced, we used the Matthews Correlation Coefficient to better assess the overall accuracy of a model.

All calculations were double checked by different persons to verify the correctness of the procedure and of the final figures. This additional work, not planned before, gave more confidence in the results, but also increased the time spent on this action.

Whenever possible statistics were also derived for a number of sub-populations allowing for a better appreciation of the real performance of the software. This referred in particular to the population of compounds used to develop the models (commonly indicated as training set) in comparison to real new estimations obtained for compounds unknown to the software. To access this information we also contacted the software developers, but however in some cases it was impossible to retrieve it. Furthermore, some models are not based on a training-set (rule-based models), so we could not make this kind of evaluation on all the models selected, obviously. We also split the compounds according to the applicability domain (AD) (i.e. the boundaries where the estimations are considered to be reliable) when this information is given by the software. This approach is different from that used in Action 6 where the AD was re-defined according to the newly generated data instead of using the information provided by the software itself. Not all the software include an AD evaluation, so this assessment was partial.

Generally speaking the performance for the continuous endpoint can be ranked according to the following order: Water Solubility > Bioconcentration Factor > Short-Term Toxicity Testing On Fish > Acute Toxicity-By Oral Route > Short-Term Toxicity Testing On Invertebrates /Daphnia. Generally speaking the performances for the categorical endpoint can be ranked according to the following order: Ready Biodegradability > In-Vitro Gene Mutation Study In Bacteria > Carcinogenicity Study.

Deliverable 14 reported results, statistics and discussion on the 7 models evaluated for carcinogenicity studies. Deliverable 23 contains final outcomes of 9 models for BCF, 5 for Water Solubility and 8 for In-Vitro Gene Mutation Study In Bacteria. Deliverable 25 is a summary of the results on the remaining endpoints (26 models).

The practical work for this action was completed in time, D23 was achieved with a slight delay, as well as for the final deliverable of this Action (D25).

4.1.6. Action 6 (Deliverable 22, 24 and 26)

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

Beneficiary responsible: ISS

In the scope of Action 6, the applicability domain of 42 QSAR models for 8 toxicity endpoints on 9 different datasets (see Table 1) has been evaluated by ISS. The main target of Action 6 was the identification of the boundaries for best use of models and of the assessment factors. The behaviour of each model was evaluated in details by relating the errors of the prediction to the chemical identity of the wrong predictions, in order to identify possible general rules for errors useful to characterise the uncertainty of the prediction in relationship to certain areas of the method. In particular, the chemical classes which can be described by the model and the classes identifying the space of exclusion of the model were identified case by case. The analysis has been performed based on the response domains, i.e. a chemical has been included in the applicability domain of the model if it is not an outlier in terms of its response values.

A general approach was adopted, with few differences depending on whether the input data were provided as a dichotomized response (i.e. positive vs. negative) or a continuous response. In both cases the response values of the model for a specific endpoint were firstly compared to the experimental data. For endpoints characterized by a dichotomized response (i.e. carcinogenicity, mutagenicity and ready biodegradability) two separate AD analyses were performed on the subsets of positive and negative chemicals. The capacity of QSAR models to match the experimental outcome was evaluated for each chemical and the subsets were divided in two categories: chemicals whose activity was correctly predicted by the model (CLUSTER 1) and chemicals whose activity was not correctly predicted by the model (CLUSTER 2).

Table 1. QSAR models whose AD has been evaluated in the scope of ANTARES – Action 6

ENDPOINT	QSAR MODELS
Carcinogenicity	TOXTREE, HAZARDEXPERT, DEREK, LAZAR, CAESAR and TOPKAT
Mutagenicity	VEGA, T.E.S.T. and TOXTREE
Ready biodegradability	VEGA and BIOWIN
BCF	VEGA CAESAR, EPISUITE MEYLAN and CORAL
Water solubility	ACD, ADMET , WATERNT , WSKOW and T.E.S.T.
LD50	ACD, ADMET, TERRAQSAR, T.E.S.T. and TOPKAT
Daphnia acute toxicity	VEGA and TOPKAT
Fish FHM toxicity	ACD, DEMETRA, ECOSAR, ADMET, TERRABASE, T.E.S.T., TOPKAT and VEGA
Fish trout toxicity	ACD, DEMETRA, ECOSAR, ADMET, TERRABASE, T.E.S.T., TOPKAT and VEGA

For endpoints characterized by continuous values (i.e. BCF, water solubility, LD50, Daphnia and fish toxicity) the AD analysis was carried out on the whole datasets by a regression method. Each chemical of the dataset was plotted on a graphic using the experimental value (X) and the predicted value (Y) as Cartesian coordinates, and the outliers were identified by applying the Dimitrov experimental variability (0.7 log unit). If the distance between the chemical and the straight line X = Y (experimental value = predicted value) was > 0.7 the chemical was considered as an outlier (i.e. out of the applicability domain of the model), and vice versa. Also in this case the dataset was divided in two categories: non-outlier chemicals whose activity was well predicted by the model (CLUSTER 1), and outlier chemicals whose activity was badly predicted by the model (CLUSTER 2).

The following steps of the analysis coincide regardless of the endpoint which the models refer to.

To check for possible relationships between the effectiveness of QSAR predictions and the presence of specific functional classes, the datasets were submitted to the ISSFUNC module for Functional Group Identification. ISSFUNC is a group profiler, coded as a Toxtree module by the Istituto Superiore di Sanità, which can be used to screen and characterise chemicals as a basis for category formation and comparison of datasets. The different distribution of functional groups between cluster 1 and cluster 2 was analyzed for each model. To establish

which types of chemicals were included in the applicability domain of a specific model we applied a threshold of 0.50. If the frequency of matched predictions is ≥ 0.50 (i.e. the activity of chemicals endowed with a specific FG is correctly predicted in most cases), the chemical class is included in the AD of the model; otherwise (frequency of matched predictions < 0.50) the chemical class is out of the applicability domain of the model. Chemical classes inside the AD of the model but having frequencies of matched predictions close to the border line for the AD inclusion (i.e. between 0.55 and 0.50) were also identified. Moreover, the frequency of matched predictions for each FG was compared to the expected one; the null hypothesis is that there is no difference between FGs with respect to the capacity of prediction of the models. Any statistically significant decrease in the frequency of matched predictions was identified and discussed. The discussion and identification of the applicability domain for each validated model is reported in details in the deliverable 26.

We experienced no relevant problem during the course of the work, except for a delay in completing the analyses of few endpoints due to a delayed receipt of the relative input data. It implied that the deliverables 22 and 24 were completed a few months late than the scheduled deadline

4.1.7. Action 7 (Deliverable 29)

Architecture for integration of different non-testing methods for best performances and coverage of applicability

Beneficiary responsible: POLIMI

While waiting for the final results of the previous actions and in particular of Actions 5 and 6, the most important input for Action 7, we performed 4 preparatory activities:

1 – review the integrating strategies proposed in literature. We have produced a short review of methods, and also concluded that the integration is not trivial and cannot be generalized to all endpoints and all uses. The software to make the integration has to be developed for our tasks. We have also provided a short didactic material for students on the basis of the predictive QSAR methods.

2 – understand the user needs through the meetings at ECHA, EFSA, and attending conferences and workshops

3 – check how to apply a software we had developed for our internal use to provide a new chunk of information. In particular we adapted the SARpy data mining software to be a way to automatically elicit knowledge from data. This software takes the SMILES strings of a dataset with the value of the activity and generates rules that express the activity in terms of

presence of substructures. Using SARpy we developed new models for the 5 data sets of CAESAR (that provides access to complete data set and SMILES) and verified the results. In particular we found out that the new models are performing as the original ones in statistical terms and add to them a set of rules that express which and how substructures are connected to the activity. This will provide an important part in the preparation of the documentation of the model as well as a new model to insert (and possibly improve) in our architecture.

4 – decide how to prepare and use the data set. We agreed with partner IRFMN the format and division of the data sets to test all the models.

The outputs of the QSAR models compared in Action 5, together with the results of Action 6, on the applicability domain, where available in the last part of the project.

In the last 8 months of the project we developed our integrated strategies starting from BCF and mutagenicity endpoints, one a regression and the other a classification model. We devised 2 different solutions for the two cases. Finally we addressed all the other endpoints studied in ANTARES, producing other integration schemata for: carcinogenicity, LD50, fish acute tox, daphnia acute tox, ready biodegradability, and water solubility.

The action fulfilled all the expected results. At the end of the project we produced a deliverable (Deliverable 29) with the proposed integrated models for the 8 endpoints, considering only the use of free software packages. In the report we stress on the need of using a few or all of the available models, in the order as emerged from Action 5, to reduce uncertainty in the prediction. Moreover we suggest to use the results of action 6 and we provided a software package for implementing the analysis of the chemical classes. This package will be soon integrated into VEGA.

Action 7 finished in December 2012, as planned.

4.2 Evaluation

For Action 1 the interview to the laboratories suitable for REACH has been the main way to get the detailed set of information which was necessary. The laboratories are often skeptical revealing their prices to subjects which are not direct customers. Anyhow, Federchimica has been quite convincing on the utility to disclose information, which are necessary for the users, but also may increase the visibility of the participating laboratories. The interview allowed to directly understand what is offered by the laboratories, and also to assess the status of the facilities. For this reason the choice to visit the laboratories has been very useful. The detailed information established for the first time an overview of the available laboratories, the quality

of the methodologies they use (verifying the GLP conditions), the number of endpoints, and the cost.

For Action 2 the investigation allowed to get a list of quantitative scores, to evaluate QSAR models. Many parameters have been identified, and split in two series, some of them necessary for the main evaluation, and the second series useful to get further details, in case of ex equo evaluations. Anyhow, these parameters allowed to sort the models, which is the main result of the action, but in addition the parameters and the scores are useful in general.

The methodology to achieve the Action 3 target was the use of computer-based databases. The long experience of Beneficiary ISS guided the evaluation of the databases. The analysis of the databases has been done in detail, assessing not only the nature of the data, but also the format of the data, their quality and their amount.

Action 4 applied the methodology defined within Action 2. An extensive search of the existing QSAR models has been done. The methodology has been with literature search, to get papers, and internet search, to get models available from the internet. Both commercial and public models have been screened. The reference for the endpoints has been the list of endpoints as requested by the REACH legislations. Thus, many tens of endpoints have been analyzed individually, using the methodology above defined. The scoring system which was defined in Action 2 proved to be a valuable way to assess the models. As a result, the models have been not only listed, but a first evaluation has been done, classifying the most promising endpoints.

The key methodology which has been adopted has been for the validation of the QSAR models, within Action 5. While the assessment within Action 4 has been done using the scoring system defined within Action 3, a more detailed assessment has been done within Action 5 on a restricted series of models, which anyhow was more than 50 models. On these models the method we adopted for the evaluation was to predict the experimental values for the chemicals, as from the databases defined within Action 3. This allowed to verify in practice if a certain model is working or not. Indeed, the possibility to correctly predict the value is the most important feature requested from a model. If the model does not predict, the model is not useful. Maybe, after further improvement, the model may achieve better performance, but in its current status it cannot be applied. The prediction has been done on about 22,000 data-points. This has been a huge amount of work, and the methodology that we adopted was to re-calculate all values twice, by a second person . Thus, the methodology was quite robust and extensive. Still, this approach may be not sufficient, since a certain model may have used many values, that we used within Action 5. Thus, a further proof of the

predictive ability, we analyzed the results of the predictions done on the new chemicals, not used to build up the model. An even more detailed evaluation has been done taking advantage of the model capability to assess the reliability of the prediction, using the applicability domain tools which are in several cases developed within the model itself. This provided the complete assessment of the model, in general terms.

Overall, the methodology applied in the scope of Action 6 has been successful in achieving the main goal of identifying the boundaries of each model. A major drawback of the method is due to the fact that the occurrence of different Functional Groups (FGs) within a dataset of chemicals presents a huge variability, with few FGs very frequent and most FGs very rare. For this reason we applied to each dataset a minimum occurrence threshold to include in the analysis only FGs whose presence was large enough to rule out the possibility that their different distribution in the subsets of matched and non-matched predictions was attributable to random fluctuations. This need strongly reduces the number of useful descriptors, and the set of selected descriptors is not completely homogeneous among models referred to different endpoints. Another issue concerns the choice of the threshold to establish which types of chemicals are included in the applicability domain of a specific model. In this context a frequency of matched predictions ≥ 0.50 has been applied for the AD inclusion. This option is very permissive and was only chosen from a logical point of view, since any other choice would be arbitrary. Therefore, the inclusion of a specific FG in the AD of a model should be interpreted with caution, and more importance should be given to the tables reporting the frequencies of matched prediction stratified by FG, evaluating on a case by case basis if the frequency of correct predictions is satisfactory for a specific chemical class.

Action 7 explored a series of methodologies to evaluate the possibility to integrate the models. Basically, two main methods are to integrate the models in a sequence, or in parallel. We verified that the different approaches may produce better results in one case or in another, depending on the endpoint. When a certain model prevails over the others, there is no real advantage on the use of multiple models. Conversely, if the results are equally good, and the models are different, it is convenient to apply methods of consensus or hybrid models.

Task by task evaluation

Task	Foreseen in the revised proposal	Achieved	Evaluation
ACTION 1. Survey of current methods for compliance to REACH legislation.	To have a real estimation on the impact of REACH in terms of costs and animals used	Yes	We obtained a general view of the competence of laboratories (more than 20) for REACH testing.
ACTION 2. Identification of criteria for non-testing methods for REACH legislation.	To identify requirements and constraints originating from the REACH legislation which may affect the non-testing methods	Yes	We carefully searched in every document available and we obtained a quite exhaustive definition of the characteristics of an NTM for REACH purposes.
ACTION 3. Identification of suitable experimental databases/data sets for ecotoxicological, toxicological and environmental endpoints.	To select some endpoints with a good number of high quality experimental data and produce a dataset for each of them, in order to perform the evaluation.	Yes	We selected 8 endpoints and for each of them we retrieved a large dataset of well-documented data from the most suitable databases, after a complete description of their features.
ACTION 4. List of (Q)SAR models for ecotoxicological, toxicological and environmental endpoints for REACH, and their review.	To write a list of all the suitable models for REACH and make it available to all users, according to the specific endpoint they will search for. The score assigned to each model permitted us to select the best models for each endpoint.	Yes	We listed 250 models suitable for REACH and the list is available on the project website. 55 of them were selected for evaluation.
ACTION 5. Validation of non-testing methods.	To investigate the state of the art of QSARs in toxicology, giving a complete statistical evaluation of the best models for regulatory purposes, according to each endpoint selected.	Yes	We got a complete view of the reliability of each of the 55 models for each endpoint, taking into account compounds unknown to the model and those considered inside the AD by the model itself. As a result, we furnished to users the instruments to select the most appropriate and reliable model, according to their needs.

ACTION 6. Identification of boundaries for best use of models (applicability domain) and of the assessment factors.	To identify the best applicability for a safer use of the non-testing methods (for which classes of compounds a particular model is reliable or not).	Yes	For 42 models we identified boundaries related to the chemical class and the mode of action of compounds, a useful information to choose the right model even in relation to the chemical properties of the compounds and to develop a strategy for integration of methods.
ACTION 7. Architecture for integration of different non-testing methods for best performances and coverage of applicability.	To integrate different non-testing methods, achieving superior performances in order to improve and enlarge the applicability of NTM in toxicology.	Yes	The integration was defined for the 8 endpoints. In 2 cases the improving of performance in comparison with the starting models is proved by better statistical parameters.

4.3 Analysis of long-term benefits

4.3.1 Environmental benefits

The latest estimation for total number of substances to be registered under Reach by 2018 is 30,000. The substances registered already in 2010 plus those to be registered by May 2013 are about 7,000 – 8,000. Thus, the outstanding substance registrations by 2018 are ca 22,000. Assuming that all these substances will be registered, and that 50 % will be within the tonnage-band below 10 ton/y and the other 50 % in the band 10 ton - 100 ton/y, and assuming that all tests, which theoretically foresee the use of animal, to cover the endpoints have to be executed, we would come to around 10,5 mln of animals used in such tests. This is the upper limit, since waiving is also expected. In terms of costs such testing expenditure without considering the other necessary tests (f.i. phys-chem tests, costs for dossier preparation, substance identification etc.) would be around 1- 1,1 billion euro. This again is the upper limit. The results of the project ANTARES evidenced that there is a possibility to move in the direction of NTM, and in some cases we are still at a good point. The use of NTM, and in particular *in silico* methods, means huge savings of animals, time and costs for registrants and industries in general.

ANTARES project contributed to cover the gap of knowledge left by REACH legislation on the use of QSAR models for registration of chemicals. In Annex XI, in particular, the criteria of an acceptable QSAR model are listed. ANTARES focused on these four conditions,

answering to many questions derived from them. First of all, our contribution was in the assess of the scientific validity of the existing models, in terms of which models are best suitable for a specific endpoint and which endpoints are predicted with a good reliability. This is the first requirement in Annex XI. Then, we evaluated the Applicability Domain of many models, which is another requirement in Annex XI, considering chemical classes and modes of action. Moreover, we made statistics both in regression and classification manner, in order to explore the possibility to use the QSARs even for CLP requirements, as requested within the third condition of Annex XI. Finally, in order to fulfil these criteria, we improved the platform VEGA, with the enhancement of the Applicability Domain Index, the introduction of a "safety margin" for BCF models and a significant improvement of material given to the user together with the prediction. This last point is useful for the fourth and last requirement within Annex XI.

4.3.2 Long-term sustainability

Due to the results of ANTARES, we submitted a new proposal to the LIFE+ Programme: PROSIL LIFE. It can have a positive influence reducing the impact of new chemicals on the environment. Indeed, it will represent a shift in the attitude of the chemical industry, which will adopt a pro-active approach, abating the impact of the substances on the environment.

The just started CALEIDOS project is a new LIFE project which is taking advantage of the results obtained within ANTARES. It is expected that it will show in which cases the non-testing methods could have been used as valid alternative to traditional methods. Thus, it will represent a way to demonstrate a possible reduction of the economic cost for the compliance to the regulatory requirements.

CALEIDOS will also promote the use of non-testing methods, which refers to a society issue, related to the use of laboratory animals. Very recently, in March 2013, the Cosmetics Regulation adopted the complete ban of animal testing for cosmetics. This shows the high interest of the European society to this issue. CALEIDOS will identify possible solutions to the problem of replacing animal testing also for other sectors, such as cosmetics.

4.3.3 Replicability, demonstration, transferability, cooperation:

ANTARES paved the way to a series of related applications affecting other sectors, besides industrial chemicals. A clear example is the Cosmetic Industry. We already explained that this industrial sector has to face the ban of the animal testing, active in Europe. The results of ANTARES have been shown in 2013 to L'Oreal, to UNITIS (a French association of cosmetic industries) and to Cosmetics Europe. All these industries were very interested in the results obtained by ANTARES.

Even if not pressed by strict regulatory requirements, other sectors will benefit from the use of in silico methods. In the pharmaceutical field, the EMA (European Medicines Agency) recently allowed the use of QSARs in the assessment of "non-genotoxicity" for impurities in drugs. So, we can expect an increasing use of software in drugs toxicology over the next years. Similarly, the pesticide industry is quite interested on in silico methods, when planning new compounds, or assessing transformation products and metabolites.

4.3.4 Innovation and demonstration value:

With the ANTARES project, for the first time, we measured in quantifiable terms the efficiency of *in silico* NTMs, giving concrete reasons on how and in which cases the use of models could be performed or not, according to the endpoints and to the chemical features of the compounds. The high level of innovation is demonstrated by the interested that our project obtained within the national Italian authorities. The Competent Authority for REACH (Ministero della Salute) supported the in silico platform VEGA, produced within ANTARES, and the logo of Ministero della Salute is present in the VEGA web site (www.vega-qsar.eu). Furthermore, also the Ministero dell'Ambiente e della Tutela del Territorio e del Mare supports VEGA, and the logo of this Ministero is also present, as well as the logo of its scientific agency, ISPRA.

The national Authority for REACH also established a working group on the use of QSAR models for REACH, coordinated by Beneficiaries IRFMN and ISS, and with the participation of Beneficiary POLIMI. A web site of this working group has been created, to promote the use of QSAR models for REACH (http://www.smart-reach.net/).

The funding of the CALEIDOS project, which is continuing and improving the work done by ANTARES, is a demonstration of the successful perspective initiated by ANTARES.

4.3.5 Long term indicators of the project success:

The indicators of the successful ANTARES project are represented by the CALEIDOS project and the working group on QSAR established by the Ministero della Salute, in which 10 different laboratories in Italy started to provide qualified consulting to industries on hazard assessment with QSAR models. In the AfterLIFE plan we provide more details. On the basis of preliminary contacts, we estimate, for the year 2013, to provide QSAR assessment on about 100 compounds from almost 20 different companies. The VEGA web site represents another living community, which is taking benefits from ANTARES. About one thousand users exist world-wide, and their number is constantly growing. New versions of the software are introduced quite frequently, adding new modules. We foresee at least 2 new releases by the end of 2014.

4.4 Dissemination issues

Action 8 was managed by FEDERCHIMICA that appointed a dissemination manager as supervisor. During the Action 8, a list of stakeholders of interest was compiled and those chosen were consulted through interviews, to identify their needs and barriers on the use of non-testing methods. Initiatives and measures to improve acceptance and dissemination included:

- the definition of a dissemination plan;
- the realization of notice boards;
- the production of information brochure, newsletters, scientific papers, didactic material and layman's report at the end of the project;
- seminars at ECHA;
- the organization of a final workshop to present the project results;
- the participation to international events on NTM;
- the practical dissemination and testing with industries.

The **action 9** produced and kept updated the ANTARES web portal (www.antares-life.eu), containing the following sections:

- on the ANTARES project;
- a discussion and list of NTM;
- didactic material for students;

- material for beginners;
- information on the events within ANTARES;
- repository on the Reports and Deliverables from ANTARES;
- links to relevant sites;
- database with results of the validation process of non-testing methods;
- FAQ;
- forum.

4.4.1 Dissemination: overview per activity

Action 8. **Communication & dissemination initiatives.**
Beneficiary responsible: FEDERCHIMICA

As planned, in September 2010, the list of end-users was produced (Deliverable 3) and used for the dissemination. It covered a broad range of stakeholders in the area of :

- Industry and Industry associations, Italian and some European

- Laboratories

- Regulatory authorities and governmental offices

- Academic structures

In March 2011, Federchimica supported by Centro Reach S.r.l. produced a first report (*Deliverable 15*) on findings coming from a stakeholder survey to identify the perceived benefits, barriers and needs in relation to the regulatory use of NTM methods, with specific focus on the their possible use already during the Reach registration process. In 2012 among the stakeholder consultation Federchimica supported by Centro Reach S.r.l. contacted further parties (a industry, a registration consultant and two laboratories) in addition to the first round investigation.
Overall, Federchimica could yield 19 responses: 10 from Industry, 5 from registration consultants , 2 from officials and 2 from laboratories.
Three questionnaires were developed and addressed the 4 different categories:

1.For companies with the aim to understand if NTM methods were already used for dossier preparation and their views on benefits and barriers of those methods.

2.Targeted to registration consultants designed to see if they had opportunity to use and evaluate NTM methods, emphasizing benefits and barriers.

3.For officials to check how NTM methods were perceived by the national authority and their acceptance in dossier preparation.

4.For laboratories, suppliers of data for companies which need to test substances for registration purposes.

Industry responses indicated that their current use of NTM like *in silico* was essentially aligned with regulatory expectations, since industry had to match the registration requirements. This is the main barrier today, together with a still limited number of available NTM methods.

Industry and consultants identified several areas (physical-chemical, toxicological + eco-toxicological) for evaluation of compounds as potential targets for the NTM methods, however some national authorities expressed concern on the legitimate use of NTM for the physical-chemical area.

It clearly appeared that NTM methods would be more important and of broader applicability in the future if regulatory acceptance was assured/certified. Here the industry statement was unanimous.

All categories expressed need for "more information or regulatory guidance". On the basis of these answers, and due to the fact that regulators are the primary actors in acceptance of NTM models, initiatives should address "way-forwards" to match regulatory information requirements. A good model alone is not sufficient.

More detailed information can be found in *Deliverables 15 (Summary on the needs of stakeholders, barriers and initiatives to improve acceptability of non-testing methods").*

In 2010 the ANTARES notice boards were prepared in five large copies to improve the visibility of the project and exposed at the sites of the 5 Beneficiaries. As before-mentioned, Action 8 also provided the publication of Newsletters. Two numbers of the ANTARES Newsletter, in English, were published: October 2010 and July 2011.

Also the ANTARES Brochures in English and Italian (more than 300) were printed and distributed. The brochure was realized in German too, even if that was not printed in more copies for distribution.

The board, the brochure and the newsletters are available for download in the Resources Area of the ANTARES web portal. (see Action 9 below)

Visits included meetings with European authorities, such as ECHA, EEA, EFSA, and Member States authorities. In particular Italian authorities were very interested in ANTARES

and promoted an Italian network of laboratories on QSAR, coordinated by the Coordinating Beneficiary, Beneficiary ISS acts as Vice-coordinator, and Beneficiary POLIMI was also involved.

Moreover, in September 2011 E. Benfenati met Frauke Stock from UBA (German authorities for environment), in Leipzig and in October 2011 he took part in the"6th Meeting of the OECD QSAR Toolbox Management Group", held in Berlin. In that occasion, he presented VEGA platform obtained in collaboration between ANTARES and ORCHESTRA projects.

ANTARES was mentioned in about 70 events, such as courses, conferences, workshops. This table shows the events, divided in 8 categories, in which ANTARES has been promoted.

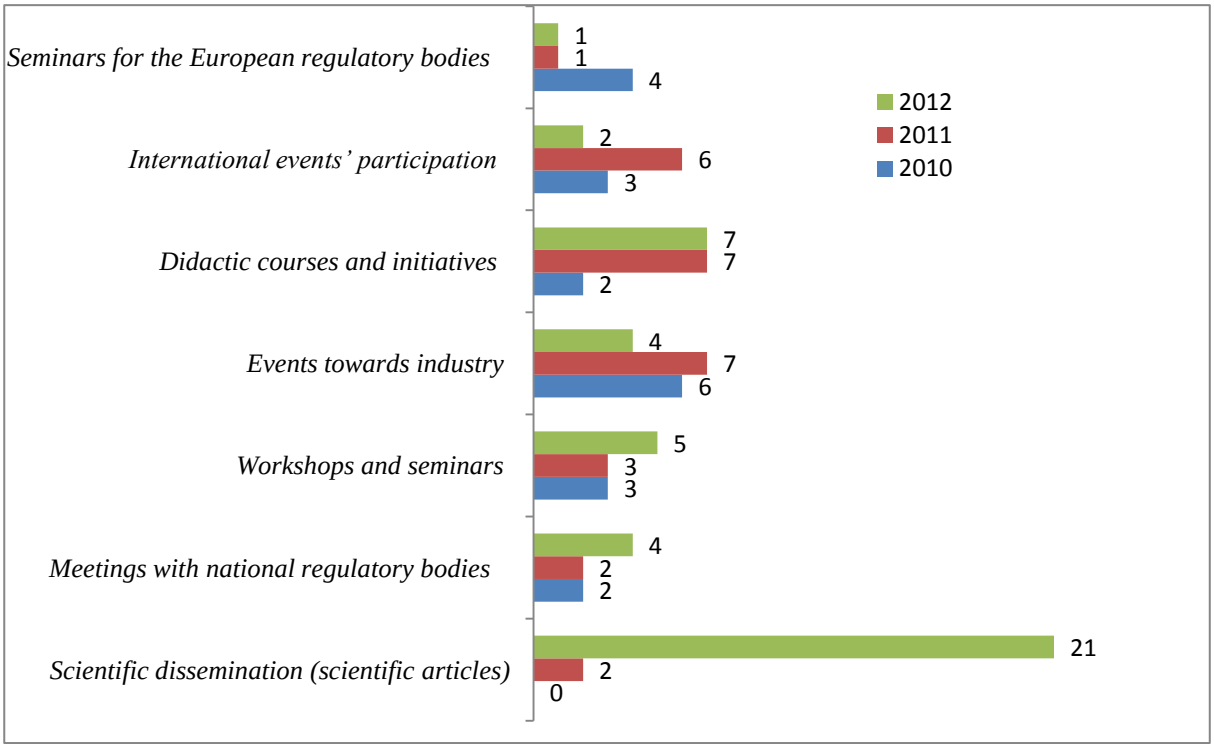

Table 1: *ANTARES events divided in 8 categories.*

As reported in *Deliverable 10 (List and description of the dissemination activities),* we started from the very beginning with the project dissemination succeeding to participate in numerous events, maintaining a similar trend during the three years of the project.

Between October 2011 and June 2012, dissemination activities of Federchimica supported by Centro Reach and by Mario Negri Institute went on and mainly included conferences and workshops, both at national as well as international level. Main tools used during these events were direct speeches – presentations and distribution of the ANTARES brochure e.g. during "8° Conferenza Nazionale Sicurezza Prodotto - Reach " in Milan on March 2012, presentation and distribution of ANTARES brochure which saw almost 500 participants.

Beside the preparation and time dedicated to these important national events Centro Reach also managed with Federchimica and with Mario Negri Institute a training course on alternative methods for Reach activities and registrations.

Within the context of international events joined, in October 2011, R. Knauf participated to the International chemical event- Chem-Med 2011, in Milan, with the presentation "How to manage test to transform regulation obligations into a business opportunity: the projects ORCHESTRA and ANTARES".

Then Federchimica joined SETAC meeting at Berlin in May 2012 also for ANTARES partner meeting. In that occasion, E. Benfenati, G. Gini and E. Boriani held a course on the practical use of the VEGA models.

Overall, ANTARES was presented at 13 scientific international events.

The list of these events is available at the ANTARES web site, where it has been updated regularly.

Didactic material was produced and is available at the ANTARES web site (see below, Action 9). An e-learning section is present there with an e-book (of about 50 pages), PowerPoint presentation, links to useful sites.

Overall, ANTARES produced 23 scientific papers. In addition to these just mentioned, other articles are going to be submitted.

Federchimica also dedicated time to ANTARES project among the Italian National Working Group for QSAR methods of the Reach Competent Authorities in Italy which involves Authorities and all in regulatory involved stakeholders like industry, industry associations, distributor associations, representatives of SMEs, Academia and R&D Institutes. This group activity is particularly pushed by E. Benfenati of Mario Negri Institute. In September 2012 E. Benfenati and other experts joined a Workshop at Istituto Superiore di Sanità, in Rome. In that occasion QSAR methods were discussed within the REACH Regulation context.

The ANTARES workshop took place on 21 and 22 November at Mario Negri Institute. The program is available on the specific ANTARES workshop website (workshop.antares-life.eu).

The presentations given mainly focused on the results of the several QSAR models used to predict the physico-chemical, environmental, ecotoxicological and toxicological endpoints listed in action 4. The material of the workshop was distributed to participants via USB keys instead of DVDs, mostly because we thought they were more adequate, welcome and user-friendly media, and in part due to financial issues.

On 23 November also a course on VEGA for QSAR and read across for industries was held by E. Benfenati. The agenda is available for download on the ANTARES workshop website.

During the ANTARES project, we also had some contacts with the media. The italian TV program “Superquark” made a report on the alternative methods with shots at the IRFMN and E. Benfenati in 2012 made an interview for the Italian scientific magazine “Focus”, talking about NTMs.

List of the events

Seminars with the European regulatory bodies:

September, 13, 2012,Rome, Italy

Workshop: Metodi (Q)SAR, REACH e il Gruppo di Lavoro Italiano

Presentations given by E. Benfenati and R. Benigni.

September, 13, 2011, Copenhagen, Denmark

Meeting at European Environment Agency (EEA)

Participation: E. Benfenati & G. Gini

October, 27, 2010, Parma, Italy

Meeting with EFSA, and presentation of the project ANTARES

Participation: E. Benfenati, A. Roncaglioni & G. Gini

September, 23, 2010, Helsinki, Finland

Meeting at ECHA, discussion about possible collaboration for joint activities on read-across

Participation: E. Benfenati

June, 29, 2010, Helsinki, Finland

Discussion at ECHA about ANTARES, with plenary talk

Participation: E. Benfenati (with A. Roncaglioni, R. Knauf, G. Gini & F. Lemke) with the talk *"Towards a safer and more transparent use of QSAR models for toxicity prediction*"

June, 28, 2010, Helsinki, Finland

Meeting with ECHA

Participation: E. Benfenati, A. Roncaglioni, R. Knauf, G. Gini & F. Lemke

International events' partecipation

June, 18-22, 2012, Tallinn, Estonia

QSAR2012

Participation of E. Benfenati, G. Gini, N. Golbamaki Bakhtyari, R. Gonella Diaza, with the posters:

- *Comparison of the Results of QSAR Models for Mutagenicity* (C. Milan, A. Cassano, N.Golbamaki Bakhtyari, A. Roncaglioni, E. Benfenati)
- *Comparison of the Results of QSAR Models for Bioconcentratio Factor* (A. Lombardo, R. Gonella Diaza, E. Benfenati)
- *Comparison of the Results of QSAR Models for* LD_{50} (C. Milan, A. Esposito, A. Cassano, R. Gonella Diaza, E. Benfenati)

- Presentation by G. Gini: "*Automatic knowledge extraction from chemical structures: the case of mutagenicity prediction"*
- Presentation by E. Benfenati: "*An integrated model for ready biodegradability for regulatory use"*

May, 20-25, 2012, Berlin, Germany

SETAC Europe 22nd Annual Meeting - 6th SETAC World Congress

- Presentation by E. Benfenati: "*VEGA, a new platform combining QSAR and read across for the prediction of chemical properties".*
- Poster: *"Collection and Screening of QSAR Models for REACH"* (C. Milan, E. Benfenati, A. Roncaglioni, R. Gonella Diaza, A. Cassano, A. Lombardo, A. Golbamaki Bakhtyari)
- Course: *Use of QSAR models for risk assessment: practical use of the VEGA models* (E. Benfenati, G. Gini and E. Boriani as teachers)

October, 26-27, 2011, Berlin, Germany

6th Meeting of the OECD QSAR Toolbox Management Group

Participation: E. Benfenati, with a presentation of the VEGA platform

October, 17-18 , 2011, Leipzig, Germany

Expert Meeting of EUROECOTOX on the use of alternative methods

Participation to the working groups: E. Benfenati

September, 3-7, 2011

Maribor, Slovenia

CMTPI 2011 - 6th International Symposium on Computational Methods in Toxicology and Pharmacology Integrating Internet Resources

Participation: A. Roncaglioni (together with A. Manganaro, R. Gonella Diaza, A. Lombardo, M. I. Petoumenou, G. Gini & E. Benfenati) with the platform presentation *"The development of new tools towards a better exploitation of the applicability domain of QSAR models"*

August, 28-31, 2011, Paris, France

EUROTOX 2011 - 47th Congress of the European Societies of Toxicology

Participation: E. Benfenati, with the talk *"In silico risk assessment"*

May, 15-19, 2011, Milan, Italy

SETAC Europe 21st Annual Meeting

- E. Benfenati (with A. Roncaglioni, A. Manganaro, R. Gonella Diaza, A. Lombardo, M. I. Petoumenou), with the platform presentation *"The development of new tools towards a better exploitation of non testing methods for regulatory purposes"*
- Posters: *"Alternative non testing Methods assessed for REACH substances"* (R. Knauf , A. Roncaglioni, C. Milan, L. Attias, S. Alivernini, , G. Gini, F. Lemke, E. Benfenati) ; *"The ANTARES Project: an evaluation of non-testing methods for REACH"* (C. Milan, L. Attias, S. Alivernini, R. Knauf, G. Gini, F. Lemke)
- Course: *Use of QSAR models for REACH* (E. Benfenati E. Boriani, A. Roncaglioni, A. Lombardo and G. Gini as teachers)

April, 11-15, 2011, Paris, France

IEEE Symposium Series on Computational Intelligence

Participation: G.Gini, with the submitted paper *"Mining Toxicity Structural Alerts from SMILES: A New Way to Derive Structure Activity Relationships"*

April, 6, 2011, Istituto Mario Negri, Milan, Italy

ORCHESTRA Workshop on QSAR & REACH

Participation: E. Benfenati, A. Roncaglioni, C. Milan, L. Attias, S. Alivernini, R. Knauf, G. Gini & F. Lemke, with the poster *"ANTARES - Alternative Non-Testing methods Assessed for REACH Substances"*

May, 30, 2010, Potsdam, Germany

OpenTox Workshop "Integrating Predictive Toxicology Resources and Applications"

E. Benfenati, with the talk *"Perspective on infrastructure requirements for chemistry research in predictive toxicology"*

May, 24-28, 2010, Montreal, Canada

14th International Workshop on QSARs in Environmental and Health Sciences

A. Roncaglioni, with the poster *"Comparison and use of QSAR software to estimate Carcinogenicity"*

May , 23-27, 2010, Seville, Spain

SETAC Europe 20th Annual Meeting

- Poster: *"Use of in silico models to estimate carcinogenicity for pesticides"* (E. Benfenati)
- Course*: Use of QSAR models for REACH* (E. Benfenati, E. Boriani and G. Gini as teachers)

<u>Didatic courses and initiatives</u>

November, 26, 2012, Milan, Italy

Lesson about in silico models in toxicology: "Metodi Alternativi alla Sperimentazione Animale in tossicologia" for degree course in "Scienze biotecnologiche veterinarie"

E. Benfenati as teacher.

November, 23, 2012, Istituto Mario Negri, Milan, Italy

The Course on VEGA for QSAR and read accross

E. Benfenati as teacher.

September, 21, 2012, Padua, Italy

Master di II livello in REACH (Venice and Padua Universities)

E. Benfenati as teacher.

August, 22-29, 2012, Sao Paulo, Brasil

Course on QSAR

E. Benfenati and G. Gini as teachers.

May, 18, 2012, Milan, Italy

Doctorate program of "Politecnico di Milano"

Lesson about Computational methods in toxicology (G. Gini as teacher).

March, 24, 2012, Bari, Italy

Master II livello in: "Regolamenti REACH (EC 1907/2006) e CLP (EC 1272/2008): valore alla sostenibilità dei processi produttivi e alla tutela della salute".

Lesson: QSAR-REACH: strumenti operativi e scenari (A. Lombardo as teacher).

March, 6, 2012, Milan, Italy

Course: "Il metodo QSAR e le sue applicazioni pratiche nel Regolamento REACH"

Participation: E. Benfenati

December, 2, 2011, Naples, Italy

Master di II livello in: REACH, Registration, Evaluation, Authorisation and restriction of Chemicals (CE n. 1907/2006)

Participation: E. Benfenati and G. Gini as teachers

November, 26, 2011, Copenhagen, Denmark

SETAC LCA Case Study Symposium

Course: Use of QSAR in risk assessment: practical use of the VEGA models and relationship with LCA (E. Boriani as teacher).

November, 7, 2011, Istituto Mario Negri, Milan, Italy

Corso sui Metodi Alternativi alla Sperimentazione Animale in Tossicologia

Participation: E. Benfenati, as teacher

September, 9, 2011, Padua, Italy

Master "Strategic Environmental Management"

Participation: E. Boriani, with the talk *"Use of QSAR in environmental risk assessment"*

August, 23, 2011, Copenhagen, Denmark

RECETO PhD course: Use of QSAR models - practical use of the CAESAR models for legislative purposes (e.g. REACH legislation)

Participation: E. Boriani, as teacher

July,4-8, 2011, Feltre (Belluno), Italy

2° Scuola Nazionale di Chimica dell'Ambiente e dei Beni Culturali. Il presente e il futuro

Participation: E. Benfenati, with a lecture on alternative methods

June, 15, 2010, Milan, Italy

TRISK course: "Identification and assessment of genotoxic and non-genotoxic carcinogens"

E. Benfenati gave a lecture on *in silico* methods

January, 14, 2010, Istituto Mario Negri, Milan, Italy

Lesson on QSAR models, within the Master Course in Risk Assessment and Risk Analysis

Participation: E. Benfenati

<u>Events towards the industry</u>

July, 17, 2012, F.I.S. (Fabbrica Italiana Sintetici), Montecchio Maggiore, Italy

Seminar on the use of QSAR models for toxicity evaluation.

Participation: E. Benfenati"

April, 20, 2012, S.I.S.T.E., Milan, Italy

Seminar on alternative methods for cosmetics (Regolamento 1223/09: Nuove metodologie e piattaforme informatiche)

Participation: E. Benfenati, with the presentation *"Valutazione della sicurezza degli ingredienti cosmetici: utilizzo di sistemi non-testing, in silico e read across"*

March, 8, 2012, Milan, Italy

8ª Conferenza Sicurezza Prodotti - A che punto siamo con il REACH

Participation: E. Benfenati with the presentation *"Non-testing methods dei chemicals - la piattaforma VEGA e le connessioni con i progetti ANTARES e ORCHESTRA"*

February, 16, 2012, Gent, Belgium

QSAR Workshop at ARCHE Consulting

Participation: E. Benfenati with *"Presentation on QSAR definitions and 5 OECD criteria for scientific validity + Demontration of VEGA platform"*

December, 15, 2011, Milan, Italy

2nd National Conference: Rethinking the equation REACH = Regulation Lecture: La nuova piattaforma VEGA per i non-testing methods dei chemicals (E. Benfenati)

October, 5-7, 2011, Milan, Italy

Chem-Med 2011 - The International chemical event

Participation: R. Knauf, with the presentation *"How to manage test to transform regulation obligations into a business opportunity: the projects ORCHESTRA and ANTARES"*

June, 20, 2011, Lisbon, Portugal

Training Workshop on QSAR as a Tool in Chemical Risk Assessment

Participation: E. Benfenati, A. Roncaglioni & G. Gini

March-April, 30-1, 2011, Barcelona, Spain

OSIRIS Fourth Annual Meeting

Participation: A. Roncaglioni

March, 22, 2011, Istituto Mario Negri, Milan, Italy

Centro Reach - Course on "Il metodo QSAR e sue applicazioni pratiche nel Regolamento REACH"

Participation: E. Benfenati

March, 8-9, 2011, Leipzig, Germany

OSIRIS ITS Stakeholder Workshop

Participation: A. Roncaglioni

February, 3, 2011, Milan, Italy

6° Conferenza Sicurezza Prodotti: REACH

Participation: E. Benfenati & R.Knauf with the talk *"I primi risultati dei progetti europei ORCHESTRA e ANTARES sui metodi alternativi"*

December,15, 2010, Milan, Italy

Workshop Centro Reach "Prepararsi al 2011: come realizzare attività dif R&S e servizi professionali, insieme al Centro Reach"

Participation: E. Benfenati, with the talk: *"Il progetto europeo ANTARES"* and the course *"Metodi Alternativi alla Sperimentazione Animale in Tossicologia."*

November, 17-18, 2010, Brussels, Belgium

Cefic-LRI 12th Annual Workshop on "Reduction of uncertainty Enabling Decision Making"

Participation: R.Knauf & E. Benfenati with the presentation of *"Predictive models and their appropriate use within the applicability domain"*

June, 16-17, 2010, Stresa, Italy

Workshop of ICCA/CEFIC/JRC "Integrating New Advances in Exposure Science and Toxicity Testing: Next Steps"

Participation: E. Benfenati & R. Knauf, with talks with international regulators and industry

May, 11, 2010, Istituto Mario Negri, Milan, Italy

Centro Reach - Course on QSAR methods

Participation: A. Roncaglioni

February, 24, 2010, Milan, Italy

Workshop of Federchimica "The LRI Long Range Research Initiative"

Participation: E. Benfenati, with the talk *"New development in predictive computational models for toxicity of chemicals"*

February, 9, 2010, Istituto Mario Negri, Milan, Italy

Centro Reach - Course on QSAR methods

Participation: E. Benfenati

Workshops and seminars

November, 21-22, Istituto Mario Negri, Milan, Italy

The final WORKSHOP of the ANTARES project: *the results of the ANTARES LIFE+ project about QSAR and Read Across for REACH*

June, 5, 2012, Milan, Italy

Workshop: 20 Anni del Programma LIFE+

Presentation of the ANTARES project by E. Benfenati

May, 30, 2012, Milan, Italy

Seminars for students and researchers of "Istituto di Ricerche Farmacologiche Mario Negri Milano

Lecture: Una piattaforma pubblica per predire la tossicità dei composti chimici (E. Benfenati)

May, 25, 2012, Brescia, Italy

Workshop: Ambiente, Energia, Sostenibilità nei progetti LIFE italiani

Presentation of the ANTARES project by E. Benfenati

May , 4, 2012, Pomezia, Italy

Conference: Il regolamento sui prodotti cosmetici

Presentation: *Integrazione del metodo read across con strumenti computazionali* (N. Golbamaki Bakhtyari)

October, 24, 2011, Bolzano, Italy

Workshop "Incenerimento e qualità dell'aria"

Participation: E. Benfenati, with the talk *"Modelli per la valutazione della tossicità ambientale"*

May, 7, 2011, Pavia, Italy

Conferenza InterMaster. Caratterizzazione tossicologica delle sostanze chimiche: prepararsi al futuro con l'esperienza maturata nel 2010 per il REACH

Participation: E. Benfenati, with the talk *"I modelli in silico: (Q)SAR"February*, 21-25, 2011, Milan, Italy

Risk assessment. Metodi di valutazione per l'ambiente e per l'uomo. Settimana Ambiente 2011

Participation: A. Roncaglioni, with a demonstration of the CAESAR software

November, 26, 2010, Milan, Italy

Workshop "Toxicity testing in the 21th Century and Alternative Method".

Participation: E. Benfenati, with the talk *"how reliable are in silico methods for toxicity prediction?"*

November, 2, 2010, Copenhagen, Denmark

Symposium: Drug Discovery and the roles of Informatics.

Participation: E. Boriani as lecturer"

October, 1, 2010, Lana (Bolzano), Italy

Workshop "La salubrità ambientale: nuovi scenari scientifici e nuovi adempimenti alla luce della regolamentazione REACH"

Participation: E. Benfenati, with the talk *"La regolamentazione REACH e l'uso dei modelli predittivi per la tossicità"*

Meeting with national regulatory body

July, 19, 2012, Rome, Italy

Meeting with representatives of the Italian Ministero dello Sviluppo Economico, Ministero della Salute, Ministero dell'Ambiente, within the Italian Gruppo di lavoro sui metodi QSAR per il REACH.

May, 18, 2012, Berlin, Germany

Meeting at Bundesinstitut für Risikobewertung (BfR) on the ANTARES project.

Participants: E. Benfenati, G. Gini, F. Lemke

February, 24, 2012, Istituto Mario Negri, Milan, Italy

Meeting of the Italian "Gruppo di lavoro sui metodi QSAR per il REACH"

Participation: E. Benfenati

February, 3, 2012, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Rome,Italy

Workshop on the use of QSAR models

Participation: E. Benfenati as teacher

November, 22, 2011,Umweltbundesamt (UBA), Dessau-Roßlau, Germany

Workshop "REACH & QSAR Models for Environment: Discussion based on Case Studies to improve Documentation and Acceptance"

Participation: E. Benfenati & R. Gonella Diaza, with a series of examples from the VEGA platform

September, 19, 2011, Ministry of Health, Rome, Italy

First meeting of the Italian "Gruppo di lavoro sui metodi QSAR per il REACH"

Participation: E. Benfenati & L. Attias (ANTARES is taken as a good source for the identification of the QSAR models)

December, 13, 2010, Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA), Rome, Italy

Workshop on "Le attività di ricerca ISPRA nell'ambito del regolamento REACH"

Participation: A. Roncaglioni, with the talk *"I metodi di non testing per la stima delle proprietà (eco)tossicologiche"*

July, 23, 2010, Rome, Italy

Comitato Tecnico di Coordinamento (CTT) per il REACH

Participation: E. Benfenati, with a presentation of the results of ANTARES for the Action 1

List of scientific papers:

1. Mining toxicity structural alerts from SMILES: A new way to derive structure activity relationships. Ferrari T, Gini G, Golbamaki Bakhtyari N, Benfenati E, In: Proc. 2011 IEEE SSCI 2011 Symposium Series on Computational Intelligence. Proc. Symp. April 11-15, 2011, Paris, France IEEE Computational Intelligence Society, Piscataway NJ, 2011; 120-127
2. Comparison and possible use of *in silico* tools for carcinogenicity within REACH Legislation. Milan C., Schifanella O, Roncaglioni A, Benfenati E, J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2011 ; 29 : 300-323
3. The average numbers of outliers over groups of various splits into training and test sets: A criterion of the reliability of a QSPR? A case of water solubility. Toropova A P, Toropov A A, Benfenati E, Gini G, Leszczynska D, Leszczynksy J, Chem Phys Lett 2012 ; 542 : 134-137
4. CORAL: Binary Classifications (Active/Inactive) for Liver-Related Adverse Effects of Drugs. Toropov A A, Toropova A P, Rasulev B, Benfenati E, Gini G, Leszczynska D, Leszczynksy J , Curr Drug Saf 2012 ; 7 : 257-261
5. CORAL: Models of toxicity of binary mixtures. Toropova A P, Toropov A A, Benfenati E, Gini G, Leszczynska D, Leszczynksy J Chemometrics Intelligent Laboratory System 2012 ; 119 : 39-43
6. CORAL: Quantitative models for estimating bioconcentration factor of organic compounds. Toropova A P, Toropov A A, Benfenati E, Gini G, Leszczynska D, Leszczynksy J, Chemometrics Intelligent Laboratory System 2012 ; 118 : 70-73
7. Calculation of molecular features with apparent impact on both activity of mutagens and activity of anticancer agents. Toropov A A, Toropova A P, Benfenati E, Gini G, Leszczynska D, Leszczynski J, Anticancer Agents Med Chem 2012 ; 12 : 807-817
8. QSAR modeling of endpoints for peptides which is based on representation of the molecular structure by a sequence of amino acids. Toropov A A, Toropova A P, Raska I Jr, Benfenati E, Gini G, Structural Chemistry 2012 ; 23 : 1891-1904
9. CORAL: Classification model for predictions of anti-sarcoma activity. Toropov AA, Toropova AP, Benfenati E, Gini G, Leszczynska D, Leszczynski J. Curr Top Med Chem 2012 ; 12 : 2741-2744
10. CORAL: the prediction of biodegradation of organic compounds with optimal SMILES-based descriptors, Toropov A A, Toropova A P, Lombardo A, Roncaglioni

A, De Brita N, Stella G, Benfenati E , Central European Journal Chemistry 2012 ; 10 : 1042-1048

11. CORAL: Predictions of rate constants of hydroxyl radical reaction using representation of the molecular structure obtained by combination of SMILES and Graph approaches. Toropova A A, Toropova A P, Martyanov S E, Benfenati E, Gini G, Leszczynska D, Leszczynski J, Chemometrics Intelligent Laboratory System 2012 ; 112 : 65-70
12. QSAR models for ACE-inhibitor activity of tri-peptides based on representation of the molecular structure by graph of atomic orbitals and SMILES. Toropova A P, Toropov A A, Rasulev B F, Benfenati E, Gini G, Leszczynska D, Leszczynski J, Structural Chemistry 2012 ; 23 : 1873-1878
13. CORAL: QSAR modeling of toxicity of organic chemicals towards Daphnia magna. Toropova A P, Toropov A A, Martyanov S E, Benfenati E, Gini G, Leszczynska D, Leszczynksy J, Chemometrics Intelligent Laboratory System 2012 ; 110 : 177-181
14. Coral: QSAR models for acute toxicity in fathead minnow (Pimephales promelas) Toropova A P, Toropov A A, Lombardo A, Roncaglioni A, Benfenati E, Gini G, J Comput Chem. 2012, 33: 1218-1223
15. Coral: QSPR modeling of rate constants of reactions between organic aromatic pollutants and hydroxyl radical. Toropov A A, Toropova A P, Rasulev B, Benfenati E, Gini G, Leszczynska D, Leszczynksy J. J Comput Chem 2012 ; 33 : 1902-1906
16. QSAR models for toxicity of organic substances to Daphnia magna built up by using the CORAL freeware. Toropova A P, Toropov A A, Benfenati E, Gini G, Chem Biol Drug Des 2012 ; 79 : 332-338
17. CORAL: QSPR model of water solubility based on local and global SMILES attributes. Toropov A A, Toropova A P, Benfenati E, Gini G, Leszczynska D, Leszczynksy J. Chemosphere 2013 ; 90 : 877-880
18. CORAL: Monte Carlo method as a tool for the prediction of the bioconcentration factor of industrial pollutants. Toropova A P, Toropov A A, Martyanov S E, Benfenati E, Gini G, Leszczynska D, Leszczynksy J. Mol Inform 2013 ; 32 : 145-154
19. Comparison of in silico models for prediction of mutagenicity. Golbamaki Bakhtyari N, Raitano G, Benfenati E, Martin T, Young D, J Environ Sci Health C Environ Carcinog Ecotoxicol Rev, 31:45-66, 2013

20. Automatic knowledge extraction from chemical structures: the case of mutagenicity prediction. Ferrari T, Cattaneo D, Gini G, Golbamaki Bakhtyari N, Manganaro A, Benfenati E. SAR QSAR Environ Res 2013 ; 24 : 365-383
21. Integration of QSAR models for bioconcentration suitable for REACH. Gissi A, Nicolotti O, Carotti A, Gadaleta D, Lombardo A, Benfenati E, Sci Total Environ 2013 ; 456-457 : 325-332
22. Validation of quantitative structure-activity relationships models to predict water-solubility of organic compounds. Cappelli C I, Manganelli S , Lombardo A, Gissi A, Benfenati E , Sci Total Environ 2013 ; 463-464 : 781-789
23. In silico models for predicting ready biodegradability under REACH: a comparative study. Pizzo F, Lombardo A, Manganaro A, Benfenati E, Sci Total Environ 2013 ; 463-464 : 161-168
24. A new in silico classification model for ready biodegradability, based on molecular fragments. Lombardo A, Pizzo F, Benfenati E, Manganaro A, Ferrari T, Gini G. Environ Sci Technol 2013 (in press)
25. Comparison of *in silico* models for prediction of Daphnia acute toxicity. Golbamaki Bakhtyari A, Cassano A , Lombardo A, Moggio Y, Colafranceschi M, Benfenati E, Sci. Tot. Environ. (Submitted)
26. Comparison of *in silico* tools for the evaluation of mammalian acute toxicity. Gonella Diaza R, Manganelli S, Esposito A, Roncaglioni A, Benfenati E, Altex (Submitted)
27. Assessment of in silico models for acute aquatic toxicity towards fish under REACH legislation. Cassano A, Bakhtyari A, Moggio Y, Lombardo A, Cappelli C I, Colafranceschi M, Benfenati E, Sci. Tot. Environ. (Submitted)

Action 9. Web portal.

Beneficiary responsible: IRFMN

Action overview

Action 9 referred to the web site. The web site is http://www.antares-life.eu. It has been active since February 2010, with all initial information on the project, and it has been updating on the average every week. It mentions the Life program, and it includes information on the project, and the beneficiaries. A series of pages provide free access to documents, such as deliverables of the project, the list of the events, a page with e-learning, containing material for beginners, but also an electronic book on QSAR and REACH, a set of resources, FAQs. There is also the list of the 250 QSAR models for REACH, with direct links to the software.

Since August 2011 up to the end of the ANTARES project nearly 6000 contacts have visited the website. In the last year of the project, we had a substantial increase in the number of contacts to the website, as shown in the table below :

Period (2012)	N° of contacts
Jan-Feb-Mar	914
Apr-May-Jun	906
Jul-Aug-Sep	1183
Oct-Nov-Dec	1260

The ANTARES website will be active and eventually updated for 5 years after the end of the project.

A link in the website sends to VEGA, the software which makes freely available a series of QSAR models, with a simple interface for the user. VEGA has been found very user-friendly and useful during our courses, it can be accessed on-line or it can be downloaded. The downloadable version is particularly indicated for users from industries, since they do not want to send structures or information through the internet. Also regulators need to use internal software, when assessing confidential structures. About 1000 users downloaded so far the downloadable version of VEGA.

The web portal structure and features

Home section

The **homepage** has been organized to provide and overview of the project, listing the main objectives the actions. There are also three specific boxes:

- "spot on" features random animations highlighting important news or resources,
- updates to the website as well as new resources or events are listed in the "news and events" box,
- the "links" box provides users with direct links to other interesting website dealing with in silico methods.

Moreover, through the homepage menu it is possible to access to several sub sections:

- REACH & NTM gives an overview of the REACH regulation and introduces the non-

testing methods as well as their importance and use within this regulation,

- PLANNED ACTIVITIES describes all the actions in which ANTARES is organized and for each of them reports the results and links to the deliverables,
- RESULTS lists all the deliverables produced by the project, with links for the direct download if they are public; in other cases a link for requesting the deliverable or a link to the password-protected intranet section is present, in order that only certain users can have access to private information and data,
- LIFE PROGRAMME gives an overview of the LIFE and LIFE+ initiatives and provide a link to the LIFE official website,
- BENEFICIARES list the members of the ANTARES projects providing a brief description of them and explaining their role in within the project.

***Figure 1**: The web portal homepage.*

Events section

This section of the web portal lists all the events organized by the ANTARES project as well as those attended by its beneficiaries. For each event the beneficiaries are listed with information about lessons, talks, posters or presentation they eventually presented.
The events are organized by target audience:

- *Seminars for the European regulatory bodies* is a series of meeting organized at European regulators' head office to present ANTARES and in silico methods,
- *International events participation* lists all the workshops, seminars, meetings, etc. attended by the project beneficiaries,
- *Didactic courses and initiatives* lists all the didactic events organized by ANTARES beneficiaries or attended by them as teacher,
- *Events toward industries* lists the events organized specifically for industrial representatives, ANTARES beneficiaries participated to this events with talk and presentations to present the possible uses of in silico methods for regulatory purposes and their benefits,
- *Workshops and seminars* lists a series of events at national level attended by beneficiaries to present ANTARES and in silico methods,
- *Meetings with national regulatory bodies* lists the meeting organized by ANTARES with regulators at national level,
- *Project meetings* are the periodic meetings of the project.

Resources section

In order to make easily available the material produced within ANTARES, the resources section has been developed and put in the main menu of the web portal. The material has been organized in three subsections:

- *Official documents* (ANTARES boards, deliverables, newsletters, etc.),
- *Presentation,*
- *Posters.*

Moreover, the sub menu of this section provide access to "QSAR resources", a page, developed within the eLearning section, which provides links to several on line resources useful for QSAR model building.

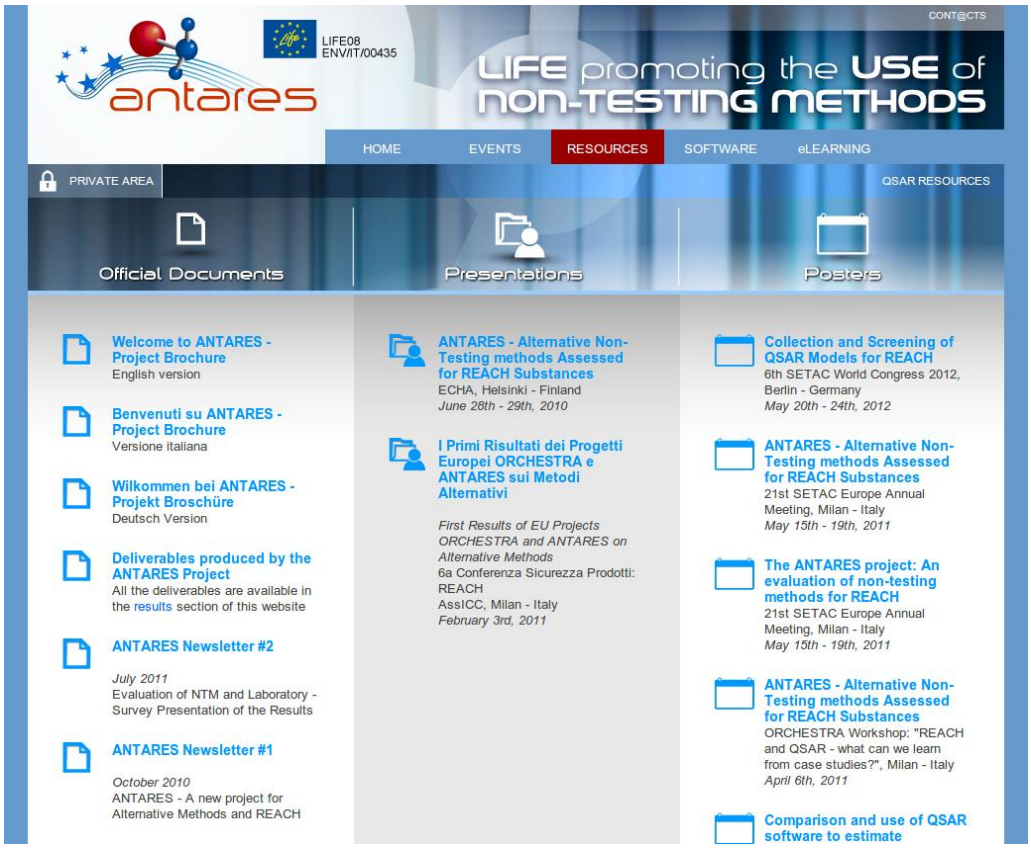

***Figure 2**: The resources section of the web portal provide links to all the downloadable material produced.*

Software section

This is probably one of the most important section of the ANTARES web portal. This page has been developed starting from the results of the action 4: *List of (Q)SAR models for the ecotoxicological, toxicological and environmental endpoints for REACH, and their review.*

The list of available software for the different REACH-related endpoints has been kept update until the end of the project and is currently composed by a total of 269 models for 38 endpoints, 93 of which are included in free software.

Due to the high number of software and endpoints, the page has been organized hierarchically and dynamically:

- The endpoints are grouped by type (physico-chemical properties, toxicological group, ecotoxicological group and environmental properties);
- The software lists for each endpoint are initially hidden, to display them the user has to click on a specific endpoint;

- For each endpoint the freely available and commercial software are listed separately;
- An option on the top of the list allows the user to choose between displaying only freely available or all the software;
- For returning visitors, an option has been add so that they can decide to display only the last added software.

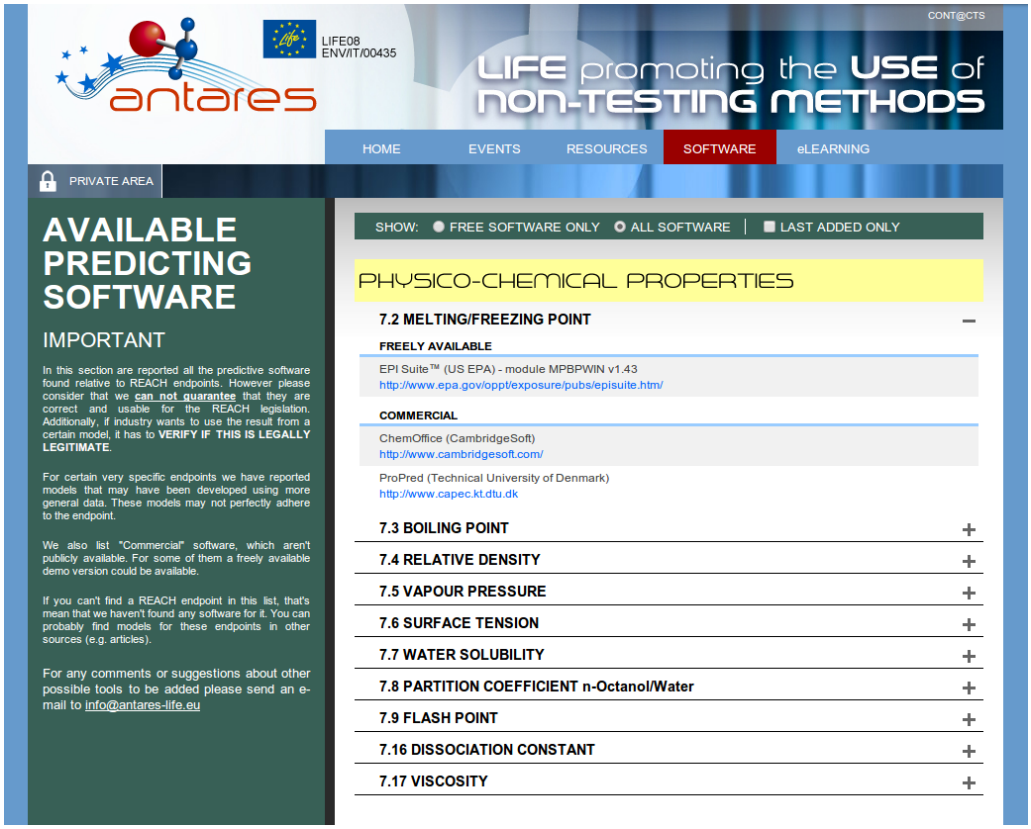

***Figure 3**: The Software section of the ANTARES web portal.*

E-Learning section

Created as a sort of mini-website within the ANTARES official web portal, the eLearning section provides material and explanations that help users entering the world of *in silico* non-testing methods.

The *home* and *updates* pages provide an overview of this section, its aims and what's new within it. Of more interest is the page *theory*, which provide complete access to the book "*CHEMISTRY, TOXICOLOGY, and QSAR: an introduction*" written by Prof. Giuseppina Gini (POLIMI) in collaboration with students of the "Alta Scuola Politecnico".

The *How to Use* page is dedicated to tutorials that drive the user through the use of available *in silico* models (VEGA and CAESAR). These tutorials have been created in a powerpoint

presentation-style, the user has to click the current slide to proceed to the next one and each slide is sided by a brief description.

The *Online Resources* section is aimed at providing access to different accessible materials and tools useful to build and test QSAR models. This section is organized in four sub-sections:

- The **Data** page provide links and descriptions of online databases that collect Biological, Toxicological and Environmental data
- the **Chemical Descriptors** page contains a list of software (with description and links) that are aimed at calculating chemical descriptors,
- the **Chemical Structures** page lists online databases that contains molecular structures,
- the **QSAR / In silico tools** page, finally, provide links to available models and tools for model development.

The Frequently Asked Question (F.A.Q.) has become more and more used within website and has been included in the eLearning section. It contains answers to some common questions about both *in silico* models and REACH. Through a form present in this page, it is also possible to submit a new question to the members of the ANTARES consortium.

***Figure 4**: The How to Use page present in the eLearning section of the ANTARES web portal.*

The private area

Official documents, deliverable with private information and data as well as templates for deliverables and reports and other material useful for the project's beneficiaries have been made available on line in a password-protected section of the ANTARES website.

The material in this section has been organized in the following categories:

- **Official Documentation of the Project**
  - Contractual Documents
  - Letters from EC
  - Minutes of project meetings
  - Deliverables
  - Reports
- **Graphic material & resources**
  - Beneficiaries' presentation templates
  - ANTARES, LIFE & BENEFICIARIES' Logos
  - Official ANTARES font
- **Presentations and material from the 6th SETAC World Congress (Berlin, May 20th-24th, 2012)**
  - Presentations
- **Materials from the 3rd Periodic Meeting & 2nd Monitoring Visit (Milan, October 10th-11th, 2011)**
  - Reports
  - Presentations
- **LIFE+ Project Administration Tools**
  - Common provisions for LIFE+ projects
  - Last updated version of Common provisions for LIFE+ projects
  - Reporting templates
  - Amendments
  - Partnership Agreements
  - Monitoring Indicators
  - Timesheets

As for the software sections, the documents in each category are initially hidden in order to make the page more compact and readable, each category is therefore provided with a “show content” button.

***Figure 5**: The ANTARES web portal Private Area.*

The Contacts section

This page displays contact information about the lead beneficiary of ANTARES (IRFMN). A map of Milan with IRFMN highlighted on it and a brochure about the city and how to reach it and the institution, are also present in this page.

The workshop-dedicated mini-site

In order to provide detailed information and help the participants to the ANTARES Workshop (November 21st - 22nd 2012) a mini website was specifically developed and published on line using the URL http://workshop.antares-life.eu/.

The **homepage** provided general information about the workshop aims, targets and topics. A small news box was also present as well as a ANTARES-related box with a brief presentation of the project.

Information on how to register to both the workshop and the course on in silico methods were present in the **register** section.

The detailed topics and the presentation titles and organisation were published in the **Programme** section, a link to a downloadable PDF of the programme was also present in this page.
The **Venue** section contained information about Istituto Mario Negri. A maps of Milan with the venue highlighted was present and a there was also a link to an informative brochure about the institution, how to reach Milan, public transportation and possible accommodations.
There was also a section about the **Course** on in silico methods, with the detailed programme.

4.4.2 Layman's report

These are the links (available in the ANTARES website) for the three versions of the Layman's report (English, Italian and German):

http://www.antares-life.eu/files/antares_layman_report_eng_update.pdf
http://www.antares-life.eu/files/antares_layman_report_ita_update.pdf
http://www.antares-life.eu/files/antares_layman_report_ger_update.pdf

4.4.3 After-LIFE Communication plan

ANTARES for the first time established a unique, reproducible procedure to compare and assess a large number of in silico models on many endpoints. This provided a new basis for the scientific evaluation of the in silico models. Indeed, each property requires a separate assessment, and the assessment for a certain model has to cover different issues, such as results in prediction on new chemicals (as different from chemicals in the training set used to build up the model itself), and evaluation of the suitable applicability domain of the model. This last aspect may include different perspectives, as done within ANTARES: evaluation of the applicability domain using tools implemented within the model itself, and a posteriori evaluation done on different chemical classes.
Finally, ANTARES put new basis and insights about a possible integration between models addressing the same property.
Thus, ANTARES established an advanced and sound basis for better use of the in silico models. The perspectives for future activities based on the results obtained within ANTARES are multiple, and some are already in place.

We introduced three different levels of activities:

A) already funded ones:

B) proposals which have been submitted;

C) plans to be finalized.

At the end of ANTARES, and strongly associated to the work done within ANTARES, a new LIFE project started, called CALEIDOS. CALEIDOS is taking the legacy of ANTARES and exploiting it. CALEIDOS has three of the five Beneficiaries of ANTARES, and added five new Beneficiaries, covering now five countries (Italy, France, Portugal, Spain, and Austria).

Two are the main extensions introduced by CALEIDOS, compared to ANTARES:

1. CALEIDOS will use the properties of the chemicals as in dossiers submitted by industry to ECHA;
2. A broader number of regulators is involved.

Thus, CALEIDOS will further check the performance and possible use of non-testing methods, using the chemicals registered at ECHA. Furthermore, the European audience will be larger, involving more countries and regulators/associated bodies in these countries: Portugal, France, Austria, Italy.

Another positive indirect result of ANTARES has been the establishment of a working group on QSAR for REACH, appointed by the Ministero della Salute, national authority for REACH in Italy. On the basis of a series of contacts occurred between the Coordinating Beneficiary and the national authority, the Ministero della Salute established such a working group, aimed to support REACH and the compliance to REACH of the Italian chemical industries. The working group is coordinated by the Coordinating Beneficiary IRFMN, joined by Beneficiary ISS as second coordinator, and is composed by many universities and research centers, including Beneficiary POLIMI. This shows a success story of ANTARES, which will continue, funded by Italian authorities.

These two initiatives already demonstrate a sound basis to promote the results of ANTARES.

Beneficiary IRFMN is using in silico models to predict properties of chemical substances upon request of chemical industries.

Another possible exploitation is with a further LIFE project, PROSIL LIFE, which is now under evaluation at the EC: PROSIL LIFE is particularly addressing industry, while ANTARES and CALEIDOS have been more active towards regulators. Industry is a sensitive target, because it suffer for the fact that industry has to refer to the opinion of the regulators.

Moreover, Beneficiaries IRFMN and ISS have submitted separately two proposals on QSAR models for REACH to the Ministero della Salute. Reply is not yet decided.
Further plans exist for the future to further exploit ANTARES. Beneficiary IRFMN is promoting the results within a series of meetings with industries and their associations. He visited L'Oreal in Paris, UNITIS, an associations of cosmetic industries in Paris, and INERIS, a French regulatory body, showing the results of the project. Beneficiary IRFMN also presented the results at a representative of Cosmetics Europe in Lisbon, and to the Danish Technical University in Copenhagen. The results of ANTARES represent a mine of data and evidences, which are now disseminated, reaching a series of stakeholders.
Another LIFE project will be prepared, extending CALEIDOS to further properties.
Furthermore beneficiaries IRFMN and KM will present a new proposal to the German UBA, asking to further extend the VEGA platform to PBT assessment.
Further proposals have been prepared and are pending for final evaluation. More plans are anticipated.
The scientific role of Beneficiary IRFMN has been increased, and it has been nominated organizer of the next conference on QSAR, which will be in Milan in June 2014. This shows a series of existing initiatives and projects, funded by public bodies or private industries.
Some dissemination events, showing the results of ANTARES and the future plans for CALEIDOS, already took place in the first 3 months of 2013 and others are planned or foreseen :

February 7th 2013
Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy
Workshop with ECHA and regulators

February 13th 2013
INERIS - Institut National de l'Environnement Industriel et des Risques, , France
Visit to INERIS and talk on "REACH and QSAR: the contributions from the EC projects ANTARES and CALEIDOS"
Emilio Benfenati (IRFMN) and Giuseppina Gini (POLIMI)

February 14th 2013
UNITIS - European Organization of Cosmetic Ingredients Industries and Services, Paris, France

Visit to UNITIS and talk on "In silico methods for cosmetics and fito-ingredients"
Emilio Benfenati (IRFMN)

February 15th 2013
L'Oréal, Paris, France
Visit to L'Oréal and talk: "Validating the QSAR models"
Emilio Benfenati (IRFMN) and Giuseppina Gini (POLIMI)

February 20th 2013
Workshop: "PRODOTTO COSMETICO. Le sfide di quest'anno". Milan, Italy
Talk on: "Le iniziative CE per la ricerca a supporto del settore dei cosmetici: esempi di metodi alternativi"
Emilio Benfenati (IRFMN)

February 27th 2013
Course on Environmental toxicology of the University degree in SSCTA, Milan University, Milan, Italy
Minicourse on "In silico modelling"
Emilio Benfenati, Serena Manganelli and Giada Maggioni (IRFMN)

March 5th 2013
Toxbank Project Meeting, Lisbon, Portugal
Discussion of the possible itneractions between ToxBank and CALEIDOS projects
Emilio Benfenati (IRFMN)

March 6th 2013
Lisbon, Portugal
Contact with Cosmetic Europe representative
Emilio Benfenati (IRFMN)

March 8th 2013
Danish Technical University - Food Department, Copenhagen, Denmark

Integrated strategies to predict health and environmental risks of chemical compounds, my experience.
Elena Boriani (IRFMN)

March 13th 2013
Università degli studi di Genova, Genoa, Italy
Lesson on "QSAR models and REACH", within the Master Universitario II livello "Management of Chemicals: la normativa REACH"
Emilio Benfenati (IRFMN)

March 20th 2013
Università degli studi di Genova, Genoa, Italy
Lesson on "Practical exercises on QSAR models for REACH", within the Master Universitario II livello "Management of Chemicals: la normativa REACH"
Alessandra Roncaglioni (IRFMN)

April 18th 2013
Workshop on the 3R in toxicology, Brescia, Italy
Talk on: "Modelli computerizzati di tossicità: realtà, possibilità e limiti"
Emilio Benfenati (IRFMN)

April 19th 2013
Course: "Corso teorico-pratico di valutazione della sicurezza dei cosmetici alla luce del regolamento 1223/2009", Milan University, Milan, Italy
Lesson on: "Uso di QSAR e read across per predire proprietà di interesse tossicologico".
Emilio Benfenati (IRFMN)

In addition to this, our paper on the results of ANTARES for Mutagenicity has been shared with the Genotoxicity Task Force of the Industries European Trade Association “Cosmetics Europe” and we foresee a webinar on the final outcomes of ANTARES at the ECHA in June 2013, with E. Benfenati as speaker.
It is likely that the several dissemination activities planned after the end of ANTARES will be funded by CALEIDOS, because it directly exploits ANTARES in the first period. Anyhow, in some cases, these expenditures will be partially supported by the local organizers.

5. Annexes

List of keywords and abbreviations used:

Keywords
QSAR, REACH, Non-Testing Methods, Environmental Toxicology

Abbreviations
QSAR : Quantitative Structure-Activity Relationship
REACH : Registration, Evaluation, Authorisation and Restriction of Chemicals
NTM : Non-Testing Method
CLP : Classification, Labelling and Packaging
RMSE : Root Mean Squared Error
MCC : Matthews Correlation Coefficient
BCF : BioConcentration Factor
ECHA : European Chemicals Agency
EEA : European Environment Agency
EFSA : European Food Safety Authority
ISS : Istituto Superiore di Sanità
POLIMI : Politecnico di Milano
VEGA : Virtual models for property Evaluation of chemicals within a Global Architecture
INERIS : Institut national de l'environnement industriel et des risques
JRC : Joint Research Center
LD50 : Lethal Dose, 50%
CAESAR : Computer Assisted Evaluation of industrial chemical Substances According to Regulations
IRFMN : Istituto di Ricerche Farmacologiche Mario Negri
GLP : Good Laboratory Practice
VCI : Verband der Chemischen Industrie
SETAC : Society of Environmental Toxicology and Chemistry
CAS : Chemical Abstract Service
CCRIS : Chemical Carcinogenesis Research Information System
PBT : Persistent, Bioaccumulative and Toxic
T.E.S.T. : Toxicity Estimation Software Tool
AD : Applicability Domain
FG : Functional Group
SMILES : Simplified molecular-input line-entry system
KM : Knowledge Miner
ISPRA : Istituto Superiore per la Protezione e la Ricerca Ambientale
UBA : Umweltbundesamt
CALEIDOS : Chemical Assessment according to Legislation Enhancing the In silico Documentation and Safe use