RAINBOW

SIXTH FRAMEWORK PROGRAMME

Project no.
LSSB-CT-2005-018695

Project acronym
RAINBOW

Project title
Research on Animal and In vitro studies and Numerical methods: Bridging Opportunities through a Workshop

Instrument: SPECIFIC SUPPORT ACTION

Thematic Priority: Call on Life Sciences, Genomics, and Biotechnology for Health
LSH-2004-1.2.3-4. Workshop: How to integrate and make optimal use of animal data, non-animal data and predictions from computer-based modelling in assessing the risk of chemical compounds.

Final Activity Report

Period covered: from **1 March 2006** to **28 February 2007** Date of preparation: **17 May 2007**

Start date of project: **1 March 2006** Duration: **12 months**

Project coordinator name: **Dr Emilio Benfenati**
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Revision: *FINAL VERSION*

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Section 1. Coordinator and contractors, project objectives and major achievements during the reporting period and project execution

Coordinator and contractors

Partic. Role*	Partic. No.	Participant name	Participant short name	Country	Date enter project**	Date exit project**
CO	1	Istituto di Ricerche Farmacologiche "Mario Negri"	IRFMN	Italy	Month 1	Month 12
CR	2	Politecnico di Milano	POLIMI	Italy	Month 1	Month 12
CR	3	Karolinska Institutet	KI	Sweden	Month 1	Month 12
CR	4	Rijksinstituut voor Volksgezondheid en Milieu	RIVM	The Netherlands	Month 1	Month 12
CR	5	In vitro Testing Industrial Platform	IVTIP	The Netherlands	Month 1	Month 12

*CO = Coordinator
CR = Contractor

** "month 1 (start of project)" and "month n (end of project)"

The RAINBOW project main objective was to organize a workshop addressing the integration of in vivo, in vitro and computer based methods (in silico) to study toxic properties of chemicals.
The workshop has been aimed at regulators, academic, industrial scientists and stakeholders such as chemical industries, animal welfare experts and public organizations: about 50 participants (http://www.rainbow-project.eu/dwnload/rainbow_participants.pdf) from 14 EU and not-EU countries attended the workshop, organised within the EC funded project RAINBOW.
The workshop discussion was useful to break down barriers between scientific and societal areas, and the REACH legislation has been discussed in its implications on the integration of methodologies.
Furthermore a website it has been created (http://www.rainbow-project.eu) where it is possible to have details of the project, its aims and outcomes.

The motivation

There is an urgent need for test systems that can fill the enormous data gap for untested or insufficiently tested substances as efficiently as possible. The recent approval of the REACH regulation by the European Parliament is an important step towards solving this society request. An efficient test strategy must take into account the limitations in economic resources and testing capacity. It also has to be in line with the aim to reduce the use of animals in toxicological testing. By the year 2009 the 7th amendment to the Cosmetics Directive prohibits the use of animals for pre-marketing toxicity testing for cosmetic products in the in the EU (Directive 2003/15/EC, Official Journal L66:26-35).

The integration

Individual toxicological and ecotoxicological tests can be described in terms of their cost, validity, reliability, and sensitivity, and there is no such thing as the perfect test. If we had, for all important endpoints, tests that fulfil the criteria of low cost, sufficient sensitivity and high validity and reliability, then the scientific uncertainties inherent in testing and risk assessment could be substantially reduced. In reality every test is a trade-off between these requirements. Since every test represents

a trade-off between these aspects, we face the challenge to combine tests with different strengths and weaknesses into scientifically well-founded and resource-efficient test systems in which the tests compensate for each other's weaknesses as far as possible. The integration of the various possibilities offered by in vivo, in vitro and in silico methods is the most mature solution to the knowledge gap which is huge; integration can take advantage of the possibilities of each approach.
In regulatory applications, toxicological tests are combined into test systems. A test system contains rules for when and in what order the different tests should be performed. With the resources presently available it will be necessary to use tiered systems in which relatively simple tests are performed for all chemicals that are up for assessment, and the outcomes of these simple tests are used to prioritise substances for further, more resource-intensive testing. Furthermore, efforts toward integration should address a more flexible scheme beyond the classical tiered approach, in which in silico screening is the first step, followed by in vitro and finally in vivo. A feed-back mechanism may offer advantages, and multiple parallel inputs may increase the understanding.
In the future a large number of data, from in vivo, in vitro and in silico studies, will became available. A strategy to integrate the different methods has to keep into account the evolving situation.

The barriers and the problems

The discussion at the workshop addressed the barriers to a more efficient integration and the needs to solve current limitations.
These are some of the problems identified:

- Language: experts in different fields use terms which are typical of a given field, and in some cases the same term has different meanings in the different fields.

- Concepts: there are cultural barriers related to concepts typical of one methodology.

- Formalism: some common practices should be codified in a clear sequence of steps.

- Subjectivism: in some cases the human expert covers a central role, and the final decision is based on the individual experience, which however is not clearly codified.

Efforts requested

The discussion identified areas to be evaluated in order to improve the integration between in vivo, in vitro and in silico methods. Some of these efforts should clarify certain aspects of individual methods, allowing a better dialogue between the methods; other efforts are required to better plan the overall integrating scheme.

- To qualify and quantify uncertainties. Each method (in vivo, in vitro and in silico) should produce a result (for instance a toxicity value or class) with a given defined uncertainty. If such an uncertainty is not defined it will be very difficult to use the result of a given method for the final chemical assessment. Also the current practice is not satisfactory in this aspect.

- To improve transparency.

- To improve standardisation, achieving a common metric.

- To evaluate advantages and disadvantages of each method, in vivo, in vitro and in silico. It has not been possible, within the workshop, to explicitly list a series of features characterising the different approaches. Some common features apply to the same category of methods, but this is not always the case. Pros and cons of individual methods are related to the target of the integrated system, which may be different.

- In order to determine the predictive value of any test, we should know how results obtained from this test relate to effects on the target system, i.e. humans, and the ecosystems that the regulation intends to protect. This, however, is seldom possible. At best, we can compare the simple test to a more advanced one, and even this is a far from perfect approximation. Even a state-of-the-art test, such as a long-term animal test, provides in its turn only an estimate of effects in humans.

- To codify in mathematical terms the common scheme for risk assessment.

- To define the purposes of the integrated system.

- To define a clearer and more formally correct decision analysis, identifying criteria and acceptability of decision.

- To identify inputs, outputs and scope of the overall scheme. Different strategies are necessary for the different scopes. For instance, if the target of the integration is for regulatory purposes, attention has to be given to false negatives, and thresholds for them. If the purpose of the integration is drug development, false positives have to be minimised.

- To identifying a reference for an integrated system. Bodies, such as OECD and ECVAM, address individual methods, but the evaluation of the integrated system requires further efforts.

- To identify features for evaluation of the integrated system, such as cost, friendliness, possibility of automatism.

- To develop integrated in silico tools, which will address the overall chemical assessment, targeting ecosystems, and improving the current approach in which in silico models mimic the existing tests focussed on specific species.

Expected outcome

The expected achievements were the successful international workshop, a document summarising final results from the workshop and the list of suitable databases and software for the addressed issues now available on the web page http://www.rainbow-project.eu/insilico.html.

Section 2. Work package progress during the entire project

Introduction - general description and milestones

Several activities were planned within RAINBOW. There were activities to be carried out before the workshop, to properly prepare it (WP 1), then activities during the workshop (WP 2), and finally activities after the workshop to better disseminate and exploit results (WP 3). WP 4 refers to the preparation of the web site whose contents where updated from time to time: before the workshop it contained the call, the description of the project, with partners and clear acknowledgement of the EC contribution. At present the website summarises the aim of the Rainbow project and its results. WP 5 refers to the management activities. The project duration was of 12 months.

WP 1: Preparation of the workshop

WP 1 main objective was to identify major topics to be discussed during the workshop and main relevant groups to be involved.

Objectives

- To prepare a questionnaire for workshop to identify the main issues to be addressed at the workshop
- To compile lists of interested stakeholders
- To prepare and disseminate the call
- To select high quality presentations for the workshop

The milestones for this WP were the call for presentations and the list of accepted extended abstracts.

Activities

We produced a questionnaire and a list of potential participants to be contacted. Aim of the questionnaire was to better address the presentations given at the workshop on relevant hot topics, to solicit contributions and suggest topics for the discussion. The questions helped in assessing the status of the knowledge in the field and the needs. Questions relied on all scientific domains cited in the call. All participants to the workshop were asked to fill the questionnaire. Table 1 shows the list of topics and questions discussed by the Steering Committee (SC).

Table 1. Preliminary list of topics and questions/areas to be addressed in the workshop

Topic	Questions/areas
I. Needs, current status of animal data	Which are the needs of regulators, scientists, problem holders, animal welfare experts and citizen organizations? Which are the endpoints to be addressed? Are current endpoints suitable, or is there a need of a reformulation of some of them? Which costs, time and trends for these endpoints? Which are the possibilities to be replaced by simpler tools (here only addressing requirements to be satisfied, and not which tools, discussed later)? Which are reproducibility and uncertainty of current methods? What is data availability? What is data quality? What is data variability and uncertainty?

	What is the experience from EU and other countries?
II. Non animal data	What information can be extracted from non-animal data? What is the standardisation and reproducibility? What is data availability? What is data quality? What is data variability and uncertainty? What is the *in vivo* endpoint which can be replaced by a given *in vitro* method?
III. Computer based modeling	What information can be extracted from *in silico* methods - molecular modelling? What kind of models can be listed depending on purposes (Models for predictions and mechanistic studies, for instance)? What kind of models can be listed depending on techniques (SAR and QSAR, with linear or non-linear methods, with data mining or based on human experts, for instance)? What is availability of models (Commercial models and publicly available)? What is model reproducibility?
IV. Integration between in vivo, in vitro and in silico techniques	Which are the perspectives opened by the integration of the three areas? Which common platform should be defined? Which are the available tools for integration? Which are the existing initiatives (OECD Global Platform, JRC initiatives, USA initiatives, etc.) How to implement a mechanism to support integration of tools? Are current in vitro and in silico tools suitable to be incorporated into risk assessment of chemicals? Which are the required performances in terms of reproducibility, quality criteria etc for risk assessment? How to cut cultural and scientific barriers between different disciplines? How to establish a solid foundation of tools? What mechanism can support an optimised exploitation of EC funded projects within this general perspective?

The list of potential participants included not only scientists, but also regulators, problem holders, NGO, SME, animal welfare experts and citizen organizations as above discussed. We already included in our consortium representatives of several areas and identified many potential participants.
In WP 1 we optimised the exact definition of the targeted attendees.

Table 2: List of contacted person invited to participate at the workshop

NAME	**INSTITUTION**	**TOWN**	**COUNTRY**
Sebastian Hoffmann	EC JRC - IHCP - ECVAM	Ispra	IT
Robert Diderich	Environment Directorate OECD	Paris	FR
Guido Sacconi	European Parliament	Bruxelles	BE
Josè Castell Ripoll	Fundacion Hospital La Fe	Valencia	ES
Gerrit Schüürmann	Helmholtz Centre for Environmental Research - UFZ	Leipzig	DE
Christina Rudén	IMM - Karolinska Institutet	Stockholm	SE
Piotr Bala	N. Copernicus University	Toruń	PL

Erwin Roggen	Novozymes A/S	Bagsværd	DK
Dick Sijm	RIVM - SEC	Bilthoven	NL
Theo Vermeire	RIVM - SEC	Bilthoven	NL
Uko Maran	Tartu Ülikool - University of Tartu	Tartu	EE
Steven Bradbury	U.S. EPA - Office of Pollution Prevention - Environmental Fate and Effects Division (EFED)	Washington D.C.	US
Oscar Hernandez	U.S. EPA - Office of Pollution Prevention and Toxics - Risk Assessment Division (RAD)	Washington D.C.	US
Hella Lichtenberg-Fratè	Universität Bonn - Botanisches Institut	Bonn	DE
Marival Bermejo	Universitat de València	Valencia	ES
Leila Risteli	University of Oulu	Oulu	FI
Vera Rogiers	Vrije Universiteit Brussel	Brussels	BE
Daniela Rosche	Women in Europe for a Common Future	Utrecht	NL
Michael Jakusch	Austrian Research Centers GmbH	Seibersdorf	AT
Marco Pintore	BioChemics Consulting	Orléans	FR
Mohit Kumar	Center for Life Science Automation	Rostock	DE
Qasim Chaudhry	Central Science Laboratory	York	UK
Antonio Conto	ChemSafe SaA	Colleretto Giacosa	IT
Sergio Sciancalepore	Corriere del Ticino	Muzzano	CH
Claudius Griesinger	EC JRC - IHCP - ECVAM	Ispra	IT
Costanza Rovida	EC JRC - IHCP - ECVAM	Ispra	IT
Michel Bouvier d'Yvoire	EC JRC - IHCP - ECVAM	Ispra	IT
Agostino Letardi	ENEA - C.R. Casaccia BAS-SIC	Roma	IT
Manuela Pavan	European Chemicals Bureau	Ispra	IT
Mike Comber	ExxonMobil	Machelen	BE
Richard Phillips	ExxonMobil	Machelen	BE
Robin Ghosh	HENKEL KGaA	Düsseldorf	DE
Domenica Auteri	ICPS	Milano	IT
Elsabetta Ugazzi	Isagro Ricerca S.r.l.	Novara	IT
Sara Lamperti	Isagro Ricerca S.r.l.	Novara	IT
Manuela Hase	Karolinska Institutet	Huddinge	SE
Frank Lemke	KnowledgeMiner Software	Panketal	DE
Judith Madden	Liverpool John Moores University	Liverpool	UK
Mark Cronin	Liverpool John Moores University	Liverpool	UK
Steven Enoch	Liverpool John Moores University	Liverpool	UK
Carlo Zaghi	Ministero dell'Ambiente e della Tutela del Territorio	Roma	IT
Marjan Tušar	National Institute of Chemistry	Ljubljana	SI
Marjan Vračko	National Institute of Chemistry	Ljubljana	SI
Marjana Novič	National Institute of Chemistry	Ljubljana	SI
Natalja Fjodorova	National Institute of Chemistry	Ljubljana	SI
Hector Galicia	Springborn Smithers Laboratories - (Europe) AG Swiss Research Center	Horn	CH
Bruno Lefebvre	Syngenta	Basel	CH
Donna Jefferies	Unilever	Sharnbrook	UK
Maura Calliera	Università Cattolica del Sacro Cuore di Piacenza	Piacenza	IT
Francesca Caloni	Università degli Studi di Milano	Milano	IT
Alberta Mandich	Università degli Studi di Genova	Genova	IT

The questionnaire and the list of potential participants were discussed and then approved by the Steering Committee (SC): the meeting of the SC was hold in Milan, at the "Mario Negri" Institute, on 16 March 2006.

Table 3: The contractors present at the Steering Committee meeting

No.	*Participant organisation name*	*Short name*	*Participant*	*Town*	*Country*
1	Istituto di Ricerche Farmacologiche "Mario Negri" (coordinator)	IRFMN	**Emilio Benfenati**	Milano	Italy
2	Politecnico di Milano	POLIMI	**Giuseppina Gini**	Milano	Italy
3	Karolinska Institutet	KI	**Lars-Arne Haldosén**	Huddinge	Sweden
4	Rijksinstituut voor Volksgezondheid en Milieu	RIVM	**Robert Luttik**	Bilthoven	The Netherlands
5	In vitro Testing Industrial Platform	IVTIP	**Wilhelmina Hermans**	Alicante	Spain

The discussion within the kick-off meeting regarded the definition of the issues to be presented, the possible invited speakers, and practical aspects of the organization.
The likely list of speakers and topics in the first hypothesis was the following:

1. REACH: scientific issues related to risk assessment (RA). Environmental RA.
2. Human RA. Someone from ICPS-WHO.
3. EPA experiences.
4. ECVAM experiences in the use of alternative methods in the tier approach.
5. ICVAM experiences in the use of alternative methods in the tier approach.
6. OECD experiences on alternative methods.
7. In silico tools for REACH. Validation, standardisation, reproducibility.
8. ECB activities for a decision support system.

During the meeting of the SC the activities more suitable for the workshop were also better defined. It was decided to promote the discussion, avoiding a simple list of presentations.
The defined main target of the workshop was to promote a debate on the way to integrate in vivo, in vitro and in silico tools aiming a sound risk assessment.
The planning of the workshop was discussed and it was suggested to start in the afternoon of the first day, with key presentations.
For the second day of the workshop, it was decided to split the activities in three sessions, to promote discussion: the first session on in vitro alternative tools, the second one on in silico tools and the third one on their integration within a unified architecture. After the three separated sessions, a final session on the third day was planned to define and agree the conclusions and results of the discussions, to be summarised into a unique draft document. The workshop should finish at lunch of the third day.
During the meeting the dates of the workshop were decided: from 11 to 13 December 2006, at the "Mario Negri" Institute in Milan.
For the publication of the paper summarising the discussion and contributions from the workshop, ATLA has been suggested and selected.
It was also defined that fellows for fellowships would have to be selected on the basis of their CV. There was a discussion about the background of the candidate fellows, allowing participation of persons with a background not only scientific, but in any case related to the application of REACH criteria for industrial chemicals.

Objective of this WP was also to start to advertise the workshop with general tools, including the web: the website http://www.rainbow-project.eu/ was created, containing information about the workshop, the project, the possibility to participate and the availability of the fellowships.

The workshop was timely disseminated in the following events:

Workshop "EU funded research on alternatives: stocktacking from FP6 and views for the future", 13-14 June 2006, Brussels, Belgium;

CASCADE course in Philosophy of Risk in Practical Risk Assessment, 9-13 October 2006, Royal Institute of Technology, Stockholm, Sweden;

Euro QSAR 2006, 16th European symposium on quantitative structure-activity relationships & molecular modelling, Italy, 10-17 September, 2006;

Further advertising was ensured through contacts within the consortiums of the EC projects CAESAR (on REACH) and CHEMOMENTUM (on QSAR), and through individual mails to tens of candidate participants (see WP 2).

Deliverables List for WP 1

Del. no.	Deliverable name	WP no.	Date due	Date of preparation	Actual delivery date	Estimated indicative person-months (*)	Used indicative person-months (*)	Lead contractor
D1.1	Questionnaire on important issues to be addressed	WP 1	Month 2 (04/2006)	Month 2 (04/2006)	Month 12 (02/2007)	0.80	1.00	P2
D1.2	List of invited participants	WP 1	Month 2 (04/2006)	Month 3 (05/2006)	Month 12 (02/2007)	0.10	0.10	P2
D1.3	Call for presentations	WP 1	Month 2 (04/2006)	Month 3 (05/2006)	Month 12 (02/2007)	1.00	1.10	P2
D1.4	List of reviewers	WP 1	Month 3 (05/2006)	Month 3 (05/2006)	Month 12 (02/2007)	0.10	0.10	P2
D1.5	List of accepted extended abstract	WP 1	Month 7 (09/2006)	Month 7 (09/2006)	Month 12 (02/2007)	1.00	0.70	P2
D1.6	Definition of the organisation local details	WP 1	Month 6 (08/2006)	Month 6 (08/2006)	Month 12 (02/2007)	1.00	1.90	P2
D1.7	Satisfaction questionnaire	WP 1	Month 6 (08/2006)	Month 6 (08/2006)	Month 12 (02/2007)	0.10	0.10	P2
						4.10	**5.00**	

(*) INCLUDING, IF AVAILABLE, PERSON-MONTHS NOT COVERED BY THE RAINBOW GRANT FOR P1 (IRFMN) AND P3 (KI)

Milestones List for WP 1

M. no.	Milestone name	WP no.	Date due	Date of preparation	Actual delivery date	Lead contractor
M1.1	The call for presentation	WP 1	Month 2 (04/2006)	Month 3 (05/2006)	Month 12 (02/2007)	P2
M1.2	The list of accepted extended abstracts	WP 1	Month 7 (09/2006)	Month 7 (09/2006)	Month 12 (02/2007)	P2

WP 2: The workshop event

Objectives

- To define the sessions of the workshop, the final list of speakers, and to schedule the sessions.
- To organise the workshop.

The milestone for this WP was the workshop.

Activities

WP2 referred to the progression and realization of the workshop itself.
The list of invited participants defined during the first meeting was used to contact relevant groups through specific individual invitations (*Table 2* shows the *list of all contacted people*), and the workshop was additionally publicised through the internet, http://www.rainbow-project.eu/, conferences and media (such as press releases).
Registration were free, to attract participants. Nine fellowships were available to young researchers. We received 7 candidates and they were all admitted to participate in the workshop (*Table 4*, list of fellows).

The SC discussed by email how to schedule the calendar, and sessions of the presentations. Participants invited to the workshop were finally:

- the partners of the consortium;
- people invited to give presentations;
- young researchers with fellowships;
- registered attendees.

Table 4 shows the list of fellows and *Table 5* the people (about 50) present at the workshop.

Table 4: List of fellows

fellows	
Anish Johnson	NSIT - Netaji Subhas Institute of Technology, Dwarka (New Dehli) - India
Harvarinder Singh	Punjab Engineering College (Deemed University), Chandigarh - India
Mark Hewitt	Liverpool John Moores University, Liverpool – United Kingdom
Silvia Lapenna	Università degli Studi di Firenze, Florence - Italy
Walkiria Alicia Levy Lopez	GSF - Forschungszentrum für Umwelt und Gesundheit, Neuherberg - Germany
Yana Koleva	Bourgas "Prof. Assen Zlatarov" University, Bourgas - Bulgaria
Zhu Yu	Gansu College of Construction Vocation and Technology, Gansu - PR China

Table 5: List of Participants at the workshop (50 attendees)

speakers	
Emilio Benfenati	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Christina Rudén	Institutet för Miljömedicin (IMM) - Karolinska Institutet, Stockholm - SE
Gerrit Schüürmann	Department Ökologische Chemie, UFZ Leipzig-Halle GmbH, Leipzig - DE
Robert Diderich	Environment Directorate OECD, Paris - FR
Robert Luttik	RIVM SEC - Expertise Centre for Substances, Bilthoven - NL
Sebastian Hoffmann	EC JRC - IHCP - ECVAM, Ispra - IT

Theo Vermeire	RIVM SEC - Expertise Centre for Substances, Bilthoven - NL
Wilhelmina Hermans	In vitro Testing Industrial Platform (IVTIP), Alicante - ES
participants	
Agostino Letardi	ENEA - C.R. Casaccia BAS-SIC, Roma - IT
Alberta Mandich	Università degli Studi di Genova, Genova - IT
Anish Johnson	NSIT - Netaji Subhas Institute of Technology, Dwarka (New Dehli) - IN
Bruno Lefebvre	Syngenta, Basel - CH
Chiara Benfenati	Universita' degli Studi di Milano, Milano - IT
Claudius Griesinger	EC JRC - IHCP - ECVAM , Ispra - IT
Costanza Rovida	EC JRC - IHCP - ECVAM , Ispra - IT
Donna Jefferies	Unilever - Safety and Environmental Assurance Centre, Sharnbrook - UK
Elisabetta Ugazzi	Isagro Ricerca S.r.l., Novara - IT
Francesca Caloni	Universita' degli Studi di Milano, Milano - IT
Frank Lemke	KnowledgeMiner Software, Panketal - DE
Giuseppina Gini	Dipartimento di Elettronica e Informazione Politecnico di Milano, Milano - IT
Harvarinder Singh	Punjab Engineering College (Deemed University), Chandigarh - IN
Héctor F. Galicia	Springborn Smithers Labs - (Europe) AG Swiss Research Center, Horn - CH
Judith Madden	Liverpool John Moores University, Liverpool - UK
Luca Lanfranconi	Universita' degli Studi di Milano, Milano - IT
Manuela Hase	Karolinska Institutet, Huddinge - SE
Manuela Pavan	European Chemicals Bureau, Ispra - IT
Mark Cronin	Liverpool John Moores University, Liverpool - UK
Mark Hewitt	Liverpool John Moores University, Liverpool - UK
Michael Jakusch	Austrian Research Centers GmbH, Seibersdorf - AT
Michel Bouvier d'Yvoire	EC JRC - IHCP - ECVAM , Ispra - IT
Mike Comber	ExxonMobil Biomedical Sciences Inc, Machelen - BE
Qasim Chaudhry	Central Science Laboratory, York - UK
Richard Phillips	ExxonMobil Petroleum and Chemical, Machelen - BE
Robin Ghosh	HENKEL KGaA, Düsseldorf - DE
Sara Lamperti	Isagro Ricerca S.r.l., Novara - IT
Silvia Lapenna	Università degli Studi di Firenze, Firenze - IT
Steven Enoch	Liverpool John Moores University, Liverpool - UK
Tialda Bouwman	TNO - N. O. voor toegepast-natuurwetenschappelijk onderzoek, Delft - NL
Uko Maran	Tartu Ülikool - University of Tartu, Tartu - EE
Walkiria A. Levy Lopez	GSF - Forschungszentrum für Umwelt und Gesundheit, Neuherberg - DE
Yana Koleva	Bourgas "Prof. Assen Zlatarov" University, Bourgas - BG
Zhu Yu	Gansu College of Construction Vocation and Technology, Gansu - PR China
Alessandra Roncaglioni	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Antonio Chana	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Chiara Porcelli	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Chunyan Zhao	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Elena Boriani	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Mosè Casalegno	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Rodolfo Gonella Diaza	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT
Vittorio Castiglioni	Istituto di Ricerche Farmacologiche "Mario Negri", Milano - IT

It was decided a proposed list of sessions:

- RA: legislation and research.
- Studies on animals, and relative data.
- Studies on non-animal organisms, and relative data.
- Computer-based models.
- Integration of the tools for RA

For each session it was decided a chairperson and *rapporteur*, with the task to prepare a summary of the topics discussed in the session.
The co-ordinator organised the event, which took place at the "Mario Negri" Institute in Milan, for three days from 11 to 13 December 2006.
The final schedule of the workshop is shown in *Table 6*. In the enclosed CD (*Appendix C*) there is further material about the workshop: abstracts of the presentations, presentations in power point, web pages, links, comments, satisfaction questionnaire, related papers.

Table 6: The Workshop Agenda

Monday, 11 December	
14:00	Emilio Benfenati ("Mario Negri" Institute, Milan, Italy) *Opening and Introduction*
14:10	Christina Rudén (The Environmental Health Risk Assessment Unit, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden) *Health Risk Assessment, REACH, and Tiered Testing*
14:40	Robert Luttik (Expertise Centre for Substances - RIVM, Bilthoven, The Netherlands) *Testing strategy in connection with the required level of confidence in regulatory hazard/risk assessment*
15:20	Coffee Break
15:45	Sebastian Hoffmann (EC JRC - IHCP - ECVAM, Ispra, Italy) *The importance of test assessment for the strategic integration of test information*
16:15	Theo Vermeire (Expertise Centre for Substances - RIVM, Bilthoven, The Netherlands) *ITS: Reaching for Integration and Intelligence*
16:45	Discussion
20:30	*Social Event*: Dinner at Sophia's Restaurant, inside the Enterprise Hotel, Corso Sempione, 91
Tuesday, 12 December	
09:30	Emilio Benfenati("Mario Negri" Institute, Milan, Italy) *In silico tools for Regulatory Purposes*
10:00	Robert Diderich (Environment Directorate OECD, Paris, France) *Qsar Application Toolbox: General Scheme, Modules and Chemical Inventories*
10:30	Coffee Break
11:00	Gerrit Schüürmann (UFZ, Leipzig, Germany) *The OSIRIS Approach on Intelligent Testing Strategy*
11:30	Wilhelmina Hermans (In vitro Testing Industrial Platform, Alicante, Spain) *IVTIP experience regarding the use of in vitro/in silico data for Risk Assessment*
11:45	Discussion
12:30	Lunch
14:15	Three Separate Sessions
	Session A1 - "*In vitro Alternative Tools*"
	Session A2 - "*In silico Alternative Tools*"
	Session B - "*Integration of Alternative Tools within a Unified Architecture*"

	Round Table A1, guided by Wilhelmina Hermans (IVTIP, Alicante, Spain), with Alberta Mandich (University of Genoa, Genoa, Italy)
	Round Table A2, guided by Uko Maran (University of Tartu, Tartu, Estonia), with Alessandra Roncaglioni ("Mario Negri" Institute, Milan, Italy)
	Round Table B, guided by Theo Vermeire (RIVM, Bilthoven, The Netherlands), with Emilio Benfenati ("Mario Negri" Institute, Milan, Italy) and Mike Comber (Exxonmobil, Machelen, Belgium)
15:30	Coffee Break
16:00	Discussions until 17:30
20:00	*Social Event*: Visit to the art exhibition "Chagall Miró" in Fondazione Mazzotta, Milan
Wednesday, 13 December	
09:30	Results of the Discussion
10:30	Coffee Break
11:00	Discussion and Major Conclusions
12:30	End of the Workshop and Lunch

The workshop event was useful to discuss the barriers, the problems of integration and the efforts requested, and to listen to different point of view and opinion.

At the end of the second day of the workshop, after the presentations, we dedicated time to develop a SWOT analysis of the three individual in vivo, in vitro and in silico methods, compared to the integrated approach.

This analysis was followed by another more careful discussion regarding how to integrate the different information and to standardized and elucidate the different methods to be pooled to catch a best quality result.

The presentations were the following:

Emilio Benfenati ("Mario Negri" Institute, Milan, Italy), *"Opening and Introduction".*
He introduced the partners of the project and the presenters to the attendance, and explained the main objectives of the project. Then he opened the workshop making a short introduction about the host organisation, the "Mario Negri" Institute for Pharmacological Research, and the idea of integration between in vivo, in vitro and in silico referred to the ongoing legislations (REACH).

Christina Rudén (The Environmental Health Risk Assessment Unit, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden), *"Health Risk Assessment, REACH, and Tiered Testing"*.
An important area in the research of Prof. Rudén is the assessment and management of risks to humans and the environment posed by the use of chemical substances. She is an expert in toxicology, environmental and health risk assessment and regulatory toxicology.
Her presentation regarded health risk assessment, REACH and tiered testing.

Robert Luttik (Expertise Centre for Substances - RIVM, Bilthoven, The Netherlands), *"Testing strategy in connection with the required level of confidence in regulatory hazard/risk assessment*".
The presentation by Dr. Luttik considered that, because the variation within a species sensitivity distribution of toxicity studies is sometimes very large, using alternative information for decision making the following should be considered: alternative information may lead to lower or higher uncertainty than test results (endpoints); dealing with uncertainty is context specific and may be different for e.g. different global regions, or different for classification and labelling purposes, or for risk assessment, etc.

Sebastian Hoffmann (EC JRC - IHCP - ECVAM, Ispra, Italy), *"The importance of test assessment for the strategic integration of test information"*.
Dr. Hoffmann has been involved in validation studies of *in vitro* test methods for several years, so he presented some of these methods. During the process of a formal validation, the animal tests for skin corrosion and phototoxicity could be replaced by *in vitro* test methods on an international level based on OECD protocol. Furthermore, just recently *in vitro* test for skin irritation were successfully validated, which might result in the replacement of the respective animal test.
However, currently this seems to be realistic only for local human health effects. He introduced some related EC founded projects. The major aim of the Integrated Project (IP) ACuteTox is to combine several of these *in vitro* test methods together with chemical information, e.g. physico-chemical properties or biokinetics. Similar use of *in vitro* models based on specific mechanisms is explored in the IP ReProTect addressing reproductive toxicity, while in the IP Sens-it-iv *in vitro* test methods for the risk assessment of potential sensitisers are developed.

Theo Vermeire (Expertise Centre for Substances - RIVM, Bilthoven, The Netherlands),*"ITS: Reaching for Integration and Intelligence".*
Dr Vermeire discussed about reaching for integration and intelligence of an Intelligent Testing System (ITS) in a case study.
He explored the integration of *in vitro*, *in silico* and *in vivo* information on skin irritating potential of a 100 chemicals considering conditional dependencies of tests, different measures of strategy assessment as well as costs. Such a study might be a helpful example when discussing ITS.

Emilio Benfenati ("Mario Negri" Institute, Milan, Italy), *"In silico tools for Regulatory Purposes*".
He underlined the new features spreading from the last and ongoing European Projects such as DEMETRA, CAESAR, CASCADE, HAIR and CHEMOMENTUM, studying in silico methods also for regulatory purposes. These projects dedicates great attention to variability and uncertainty of data, and to innovative development of new concepts of in silico tools (hybrid systems, expert systems, validation and use of final models). In silico methods offer advantages which in some cases are unique. They are mainly related to the non-animal use, low costs, and reduced time for obtaining results.

Robert Diderich (Environment Directorate OECD, Paris, France), "*Qsar Application Toolbox: General Scheme, Modules and Chemical Inventories*".
He introduced the OECD initiative to collect and make available QSAR models. The project is ongoing. A database is also under development.
The main electronic tool for data submission, evaluation and exchange used in the OECD HPV Chemicals Programme is the International Uniform Chemical Information Database (IUCLID). IUCLID is a software program for the administration of data on chemical substances. This database program was originally developed to fulfill requirements in the EU for the evaluation and control of the risks of existing chemical substances.

Gerrit Schüürmann (Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany), "*The OSIRIS Approach on Intelligent Testing Strategy*".
The EC project OSIRIS brings together European researchers to solve problems of integration on an applied level. In this step from concepts to application, communication and practicality will be of crucial. Thus a platform, where scientists, regulators and stakeholders exchange their views of a practical integration of information for risk assessment, could increase the chances of success of the ITS.

Wilhelmina Hermans (In vitro Testing Industrial Platform - IVTIP, Alicante, Spain), "*IVTIP experience regarding the use of in vitro/in silico data for Risk Assessment*".
Like for every type of information, there is uncertainty about the relevance for humans of the *in vitro* data. However, classical uncertainty factors in risk assessment can usually not be applied as in vitro data cannot directly be used for risk assessment. But also in hazard assessments, which build the base for classification and labeling of chemicals, uncertainties have to be considered. They are for

example reduced by defining applicability domain of an *in vitro* test method, i.e. the spectrum of chemistry, for which the methods produces useful results. A further uncertainty has to be considered in the test method assessment, which is usually based on the comparison of *in vitro* method results with *in vivo* method results. In this comparison the in vivo information, often from only one experiment, is usually considered as being deterministic, i.e. neglecting aspect of variability (reproducibility). Dr. Hermans underlined the industrial point of view, which is mainly practical, aimed to achieve alternative methods which can be approved and used.

The ***afternoon session of the second day*** was firstly divided in **three groups** working to assess a SWOT analysis of the following themes:

Session A1 - "*In vitro Alternative Tools*"
Session A2 - "*In silico Alternative Tools*"
Session B - "*Integration of Alternative Tools within a Unified Architecture*"

Then each session was transformed in a presentation for the rest of the public to develop a critical and larger examination on each method, creating a SWOT analysis and the key points for the discussion.

Round Table A1, guided by Wilhelmina Hermans (IVTIP, Alicante, Spain) with Alberta Mandich (University Of Genoa, Genoa, Italy).

Round Table A2, guided by Uko Maran (University Of Tartu, Tartu, Estonia) with Alessandra Roncaglioni ("Mario Negri" Institute, Milan, Italy).

Round Table B, guided by Theo Vermeire (RIVM, Bilthoven, The Netherlands) with Emilio Benfenati ("Mario Negri" Institute, Milan, Italy) and Mike Comber (Exxonmobil, Machelen, Belgium).

Focal theme of the discussion: the comparison of the different methods

It is useful to identify key features of the different methods, in vivo, in vitro, and in silico, through a SWOT analysis (strengths, weaknesses, opportunities, threats). To do this, key factors has been evaluated, such as cost, time for execution, validity, reproducibility, sensitivity, ethical factors, safety of the test executions, etc. Costs refer to several aspects, such as cost of the single value, of the apparatus, of the training of the personnel. Validity refers to the capability to measure the intended target. The issue of assessment of chemicals for human health and ecotoxicity are below summarized. Sensitivity refers to the capability to assess a small effect.

The details of the SWOT analysis for in vivo, in vitro and in silico methods are presented below:

In vivo methods

Strengths

The major strength is that in vivo methods are closest to the target, both in case of human health and ecosystem. However, it is clear that in both cases in vivo models are not sufficient. For human health, the animal model may give wrong answers, depending on the differences in the organisms and scale. Different metabolic processes apply, for instance, which may activate transformations of the parent compound. For this reason, safety factors are applied, to refer the values obtained from animals to the human situation.
Similarly, in case of ecotoxicity, only a few selected species are used, as representative of a much larger ecosystem. Thus, again, safety factors are applied to cover all different species. Furthermore,

effect to a given species may be due to an indirect activity affecting another species on the trophic chain, and this requires a wider integration of the results.
Many in vivo methods have been standardised, and this represents another clear advantage. OECD started in the late 1980's the introduction of harmonised guidelines for toxicity tests, allowing a better acceptance of data. Good laboratory practice (GLP) principles have been promoted to ensure quality and reproducibility of results.
Related to this, is that the in vivo approach is the major source of data, and so far the other models refer to in vivo data to compare results.
In vivo test are suitable to measure effects of mixtures, while other methods, such as in silico, have major problems.
Other strengths are specific to some tests. For instance, in the case of carcinogenicity assays for human toxicity, the number of false negatives (not to detect a human carcinogenic compound) using two rodent species is low.
Long-lasting experience (experimental and regulatory) has resulted in general acceptance and accumulation of knowledge about in vivo tests.

Weaknesses

Starting some twenty years ago, the society has more and more questioned the need of in vivo methods. Widespread ethical concerns have been shaping the political arena resulting in strong incorporation of Russell and Burch's 3R, i.e. replacement, reduction and refinement, into legislation, especially in the fields of cosmetic ingredients and industrial chemicals. E.g. with the 7th Amendment to the Cosmetics Directive European Union decreed a complete ban on animal testing for cosmetic ingredients as soon as alternative non-animal methods are validated by ECVAM and adopted into the EU legislation, with a maximum period of implementation until 2013 when the animal testing ban will be enforced regardless of alternative method availability.
Furthermore, animal experiments require a considerable amount of resources. While maintenance of animal facilities is laborious, some tests can last years, i.e. those studying effects over more than one generation. Indeed, major costs of the REACH legislation are related to the animal methods. A reduction of animal number is translated into a similar reduction in the costs.
Furthermore, the availability of laboratories to perform complex tests, such as multi-generation studies, may be a critical factor for the achievements of the requested results within REACH.
Another problem related to the animal model is that in many cases the dose used for the experiment is high, and this may represent an issue, in the more typical case of exposures at lower doses. However, the experiment done at lower doses would require a higher number of animals to increase the statistical power of the response, and thus typically high doses are preferred.
We already mentioned that animal model itself is not the ultimate endpoint, and animal models have some reported false negatives for the human toxicity. This has been identified mainly for pharmaceuticals, because in this case there is data on animals and humans.
Referring to ecotoxicological endpoints, there is a quite large spread of toxicity values, and this requires the introduction of safety factors, to protect a larger number of species. Indeed, it has not been possible to identify a species which consistently is the most sensitive one.
Animal models are as some windows open to the phenomenon to be studied. The methods have to be standardised, so that it has been possible to define a relatively limited number of them. Many aspects of the phenomenon are not sufficiently addressed. A review evaluated current in vivo methods and specific strengths and weaknesses for the different endpoints. There is a need of more models addressing reproductive and developmental toxicity, including endocrine chemical disruption, for the complexity of this aspect. For certain human tumours (testicular and breast) there is the need of good animal models.
Another issue is that for some endpoints, such as carcinogenicity and developmental toxicity, there is a quite high number of false positives, which can produce a loss of potentially useful chemicals.
Sensitivity can be an issue, due to the fact that a high number of animals is required to have enough statistical power.

Opportunities

Research is proceeding in the animal models. This field will not be closed due to the introduction of alternative methods. Transgenic animals offer new opportunities. These animals are animals into which cloned genetic material has been transferred. This open several fascinating perspectives to address certain diseases.
Proteomics, genomics, and metabonomics are introducing new approaches offering a completely new perspective to old problems. Impacts in toxicology are expected.
Other technologies, such as nuclear magnetic resonance, and its application in imaging, enable to investigate chemicals in animals in a non-invasive way.

Threats

The more critical factors against the use of animal models are the increased concern against the use of animals in research. The new EU legislation on cosmetics introduced a deadline for the use of animals for tests on cosmetics. More in general, there is more and more concern for the animal use in research. Thus, there will be higher restrictions in the use of animals.

In vitro methods

Strengths

In vitro methods have several advantages. They do not use animals. They are in general cheaper and faster than in vivo methods. In some cases they are indeed much cheaper and faster than in vivo methods.
In vitro methods are more suitable to mechanism studies than in vivo methods. Indeed, thanks to a simplified approach which separate different steps in the overall toxicity phenomenon, it becomes easier to identify key factors playing a major role in certain phases of the toxic biomechanism.

Weaknesses

In vitro methods do not take into account the animal complexity. Thus, several steps which play a role in the final toxicity phenomenon can escape from recognition.
The in vitro method requires a validation process.

Opportunities

Several in vitro methods are suitable to be run in parallel, and with a robotic system. Thus, in the future it is likely that batteries of in vitro models will be performed together.

Threats

No threats identified

In silico methods

Strengths

In silico methods offer advantages which in some cases are unique. They are mainly related to the non-animal use, low costs, and reduced time.
Obviously, in silico models do not use animals. They refer to previous animal experiments, or on in vitro data, in more limited cases (basically for the mutagenicity models, since the number of in vitro

data is still limited). This fact means that the quality of the in vivo (or in vitro) data is fundamental. The uncertainty of the in vivo data is a limit to the accuracy of the model. We cannot expect results from in silico methods which are better than those used to build up the model. Indeed, any in silico model has at its basis a series of experimental data. If the data is noisy, the model will be poor. On the other side, since in silico methods are statistical in their nature, they can cope with errors in toxicity data. Thus, in some cases it is possible that in silico models may spot unusual values in a series of similar compounds. Some in silico models are more robust to noisy data than others (for instance, artificial neural networks are know to be quite robust).

The cost of in silico models vary, and we should consider three factors: the cost of the software, of the computer and of the personnel. Some models are free, such as the US EPA models as ECOSAR for ecotoxicology. In Europe, DEMETRA hybrid models are a series of free in silico models to predict ecotoxicology for pesticides towards daphnia, trout, quail (oral and dietary exposure) and bee. The expert system Oncologic is due to be publicly available. It has been developed by the US EPA for carcinogenicity.

In the case of free software, the cost is null and thus the use of these models can be applicable for any chemical, regardless to their production: legislations such as REACH require different data depending on the industrial production, but the free availability of predictive tools can easily promote their use regardless of the production volume and, actually, even before the synthesis or preparation of the final chemical, industry can explore possible critical effects and redirect the studies towards less toxic analogues. However, some commercial programs are quite expensive, from several thousands of euros to tens of thousands, and this may limit they use. In some cases licenses have to be renewed every year. For instance, some docking models are in these conditions. In silico models require a computer, which typically is not a barrier. Even though the cost of the computer is decreasing, in some cases personal computers are not sufficient, and thus the cost of the computer should be added (for instance for some advanced docking programs).

Another cost may be for the experienced personnel requested for some advanced modelling programs, as in the case again of docking studies. However, the use of some easy, cheap program, require limited or very limited experience, and in some cases no specific training is necessary.

The time necessary to get the result is another important factor, which in the case of in silico model may give preference to this approach. Indeed, in some cases the prediction is almost immediate. Furthermore, some software allows to process data in batches. The Openmolgrid software obtained results for about 30,000 chemicals in about one week .

In silico methods are the only ones which do not need the synthesis or preparation of the chemical, thus the toxicity can be assessed before the chemical production and this information can be used to improve the synthesis strategy made by chemical industry.

Related to this there is the fact that in silico models are intrinsically much safer to be conducted, because the risk of chemical or biological contamination is null. Furthermore, the in silico method does not cause pollution, as the other methods (in vivo and in vitro) and do not use solvents.

Another unique advantage of the in silico approach is its world-wide availability. In some cases a simple internet access is enough, or a computer. The laboratory techniques (in vivo and in vitro) have more requirements.

In some cases in silico predictions can be obtained by non experts, through the internet, while laboratory techniques require a certain training, which can be quite complex. It has to be remembered that other in silico methods, such as docking, need dedicated training.

In silico models give results which in many cases are highly reproducible, or even exactly the same, because the results is due to chemical features which are invariants. However, in some more complex models, using tri-dimensional structures and conformation optimisation, some differences in results may be expected, due to the manual optimisation, and to the stochastic nature of the process.

In silico methods are complex, multiple and flexible. This results in a series of advantages. Some models are more suited to predict the toxic effect, while others are more indicated to highlight the toxic mechanism.

In silico methods can address multiple endpoints at the same time and address differences in the results between two endpoints. This feature is not unique of in silico methods: for instance certain in

vivo studies can provide results useful for more than one endpoint. The possibility to identify differences in endpoints results can be useful to understand species differences.
In silico methods can describe a phenomenon in mathematical terms, and thus they are more suitable to be integrated with other tools, in silico and not. We have already mentioned the DEMETRA models. Actually each DEMETRA model incorporate a battery of in silico models. This increases the model robustness and improves performances. In the case of the DEMETRA model the endpoint evaluated is the same, one of the five listed above: trout, daphnia, quail (oral and dietary exposure), and bee. However, other hybrid models have addressed different endpoints together: for instance, it is possible to predict human carcinogenicity with a combination of methods including models to predict rodent carcinogenicity. A still different approach is the integration of in silico and in vitro results. For instance, carcinogenicity has been predicted with a hybrid system using in silico and in vitro methods.

Weaknesses

In silico models do not provide an experimental result, of course. This is a major conceptual difference compared with the in vivo and in vitro methods. As we said, the in silico methods refer their predictions on the basis of the experimental values used to build up the model. Thus, the predicted value is somehow a reflex of the toxicity values of the chemicals used for the model. The meaning of the predicted value is statistical.
The in silico model requires a validation process.
The in silico approach requires a better description, training, explanation, to improve acceptability.
Only a few models are currently available.

Opportunities

In silico models, thanks to their mathematical basis, are suitable to be at the basis of an integrated approach with other methods, such as in vivo and in vitro, but also with the omics studies and research in system biology. In silico models can increase in number very rapidly.

Threats

No threats identified

Discussion

The challenge to reach a deeper, more efficient assessment of the chemicals and their toxic effect is huge. The problem is complex and the lack of knowledge impressive. For this reason, it is necessary to take advantage of all pieces of information we may have. But this requires an evaluation on the way to compare results of very different nature. The basic concept is that the information closest to the correct evaluation is that obtained with animal models. However, the second concept is that further information can be obtained from other methods, and that the value of this information is not zero. Any test can provide useful data. In this perspective it is important to optimise the possible scheme to organise in the most suitable way the different inputs. Such a process requires a careful discussion between experts of many fields. It also requires a better definition of the nature of the process and single components. This will be anyhow beneficial, also because it has been expressed a “tendency to underreport, or even omit, the details of the interpretative methodology used”. The use of “all” rather than some evidence attracts more and more the assessors. This produced several proposals of integrations. Calabrese et al. assigned greater weights to in vivo rather than in vitro studies. Menzie et al. suggested a different approach, considering the quality of the studies and other factors. In this direction in silico methods can be added, as a further source of information.

The criteria for the definition and acceptance of the proposed scheme should involve a large number of scientists. The approach of integration should report how the single components are used, and eventually allow the choice to use or not a method. This may improve the acceptance of the proposed protocol, producing in a transparent way results under different assumptions.
The way to use the possible tests into an optimised strategy should be planned thinking both to the way to use the results with certain weights, and to perform the tests in a sequential way.
The workshop event covered *social events* in the evening, like a dinner in the first day (at the Sophia's Restaurant, inside the Enterprise Hotel, Corso Sempione, 91, Milan) and, in the second evening, a visit to the art exhibition "Chagall Miró" at the Fondazione Mazzotta in Milan.

The third day was mostly dedicated to decisions and comments on the previous days.

We do not register main problems. The presence of many scientists from different field stimulated the discussion, which explored many areas. In some cases the discussion interested some technical issues, as in the case of a long discussion on QSAR and mechanistic based approaches. Different opinions where present, reflecting different schools in the in silico community . This underlines the need of this kind of events and of more work in the field.

Deliverables List for WP 2

Del. no.	Deliverable name	WP no.	Date due	Date of preparation	Actual delivery date	Estimated indicative person-months (*)	Used indicative person-months (*)	Lead contractor
D2.1	The final programme	WP 2	Month 7 (09/2006)	Month 8 (10/2006)	Month 12 (02/2007)	1.00	0.90	P1
D2.2	The list of chairmen and rapporteurs	WP 2	Month 8 (10/2006)	Month 8 (10/2006)	Month 12 (02/2007)	0.10	0.10	P1
D2.3	The workshop	WP 2	Month 9 (11/2006)	Month 10 (12/2006)	Month 12 (02/2007)	1.70	2.53	P1
D2.4	The questionnaire with satisfaction report	WP 2	Month 9 (11/2006)	Month 10 (12/2006)	Month 12 (02/2007)	0.10	0.10	P1
						2.90	**3.63**	

(*) INCLUDING, IF AVAILABLE, PERSON-MONTHS NOT COVERED BY THE RAINBOW GRANT FOR P1 (IRFMN) AND P3 (KI)

Milestones List for WP 2

M. no.	Milestone name	WP no.	Date due	Date of preparation	Actual delivery date	Lead contractor
M1.1	The workshop	WP 2	Month 9 (11/2006)	Month 10 (12/2006)	Month 12 (02/2007)	P2

WP 3: Dissemination and Exploitation

Objectives

- To disseminate the results of the workshop
- To exploit the results of the workshop

The milestones for this WP were the dissemination through journal/web and the summary of dissemination and exploitation tools.

Activities

The dissemination includes a summary of the discussion at the workshop, with main results, a list of databases, SWOT analysis for Risk Assessment (see WP 2), submitted papers, and the updated web site.

Two *papers* with references and acknowledgements to RAINBOW have been prepared and sent to journals:

- The first paper, which summarizes the project results, has been prepared with the contributions both of members of the project consortium and participants in the workshop:

  Benfenati E., Gini G., Hoffmann S., Luttik R., Rudén C.

  "The integration of in vivo, in vitro and in silico methods for the chemical assessment: problems and perspectives"

  This paper has been submitted for publication to *ATLA*, which has been chosen as host journal by the Steering Committee (SC) during the kick-off meeting (hold in Milan, at the "Mario Negri" Institute, on 16 March 2006).

- The second paper with acknowledgements to the RAINBOW project is the following:

  Benfenati E., Senese V., Lodi M., Marras R., Testa S., Finizio A.

  "Definition of an integrated ecologic risk index"

This paper has been submitted for publication to *Chemosphere*.

Both the submitted papers are enclosed, with acknowledgements to the RAINBOW project and to the European Commission, in Appendix A.

The website was updated with the report of the project, at the address http://www.rainbow-project.eu/results.html. The complete list of participants was made accessible from this web page through a link to a downloadable PDF file.
A list of suitable database and software for in silico tools was created and put available on line at the page http://www.rainbow-project.eu/insilico.html of the project web site. This list is also in Appendix C of the report.

Further dissemination is foreseen at a planned conference: Prof Christina Rudén has scheduled and organized a special session on integration of in silico, in vitro and in vivo methods for RA, to continue the dissemination started by RAINBOW. This will be a Symposium for EUROTOX 2008: the abstract of the symposium, that has been proposed with the participation of the same speakers of the RAINBOW workshop, is below:

"With the implementation of REACH, the proposed new European legislation for general/industrial chemicals, the regulatory system will have to handle test requirements for 70 000 general

chemicals. This has put scientific and regulatory focus on how testing should effectively be performed in the regulatory context. How should chemicals be selected for testing? How extensive testing should be required? What tests should be prioritized?

A regulatory test system consists of the individual tests allowed to be used, as well as the rules and criteria that determine which tests are relevant and in what order they should be performed. There is an urgent need for scientifically robust test systems that can fill the enormous data gap for insufficiently tested industrial chemicals as efficiently as possible. Such a strategy must take into account the limitations in economic resources and testing capacity. It also has to be in line with the aim to reduce the use of animals in toxicological testing.

Although regulatory testing has a long tradition, little scientific effort has been spent to systematically analyze how single tests best should be combined into efficient testing systems. One particular challenge is to incorporate new – non-animal – test methods such as QSARs and in vitro models. It is the purpose of this symposium to contribute to a scientific discussion in this new area of research".

Summary documents

Individual partner dissemination:

P1 Istituto di Ricerche Farmacologiche "Mario Negri"

Dr Emilio Benfenati, coordinator of RAINBOW, participated to the Conference "Europe Goes Alternative" on 18 December 2006 in Brussels, in order to disseminate the workshop results and inputs.
Dr Benfenati participated also to a bilateral meeting, on 5 February 2007 in Brussels, with representatives of P3 (KI), to discuss future exploitation within a new proposal, SENTINEL, for the 7th Framework Programme.

P2 Politecnico di Milano

The topic of in silico methods deserves also in the wide area of informatics. To improve the knowledge of the problems and the involvement of new actors P2 (POLIMI) prepared two main actions:

- A seminar with the title "in vivo, in vitro, in silico: dalla sperimentazione sui viventi alla sperimentazione sui robot", which has been held in four scientific high schools in Milan as part of the activity to orient students to the choice of the university. The activity has been organized through the Politecnico di Milano, Faculty of Information Engineering.

- A second activity has been proposed within the research projects of the Alta Scuola Politecnico (ASP), the school for selected students of Politecnico di Milano and Politecnico di Torino (http://www.asp-poli.it/presentation/): it consists in the presentation of an interdisciplinary project about "New chemical descriptors", a topic that has been highly debated in the workshop and that is not clear to many of the actors in the field. The project will be carried on by 5 students, and will continue until the end of 2008, with support also by the "Mario Negri" Institute.
  More details at http://www.asp-poli.it/presentation/resources/proj_book_IIICycle.pdf

P3 Karolinska Institutet

Dr Manuela Hase, Integration Manager of CASCADE, met the organizers of the RAINBOW workshop in Milan on 20 November 2006, in order to discuss actions to be taken regarding WP 3.

A list of important issues to be addressed during the RAINBOW workshop was prepared too.

Dr Ingemar Pongratz and Dr Manuela Hase, as representatives of P3 (KI), participated in a bilateral meeting (5 February 2007) with the coordinator to discuss exploitation within the new proposal SENTINEL for the 7th Framework Programme.

P3 KI involved Prof Christina Rudén, teacher within CASCADE schools organised by Karolinska Institutet: she provided a preprint version of her latest manuscript called "Towards a theory of tiered testing", accepted for publication in Regulatory Toxicology and Pharmacology.
The publication was distributed by e-mail among all the participants of the RAINBOW workshop.
Comments on the workshop report were given.

P4 RIVM and *P5 IVTIP* concentrated their main dissemination and exploitation activities within their other ongoing EC projects (see below).

Exploitations within EC projects:

Moreover partners are involved in ongoing EC projects exploiting and testing the results of the workshop within their projects.

P1 (IRFMN) is actually involved in the following projects:

- CAESAR
- OSIRIS
- CHEMOMENTUM
- CASCADE

P2 (POLIMI) will exploit results within CAESAR.

P3 (KI) will promote the outcomes of the workshop through the CASCADE portal and the meetings within the NoE it coordinates.

P4 (RIVM) will exploit results within HAIR.

P5 (IVTIP) will exploit results within several EC projects, such as Reprotect and Sens-it-iv.

Basically, the lessons from the RAINBOW workshop to be reversed into the other projects are relative to the need to:

1) Think individual methods (in vivo, in vitro, in silico) as tools which will benefit from closer integration. The improvement related to this wider perspective is expected more in EC projects which do contain those different tools, such as CASCADE and Reprotect.

2) Identify in the RA, or in the chemical evaluation in general (also for labelling purposes) the target of the output of the individual methods. This will generate a refocusing of the results of some projects, such as CAESAR, providing a clearer view of a suitable exploitation process of the results obtained by individual methods. In this way, addressing the target close to the regulatory format useful for stakeholders, the exploitation will be facilitated.

3) Characterize the results of the methods keeping in mind the need to compare integration and, at the end, standardize the results of the different methods according to a scheme suitable for easier integration and use of the result. This will require more efforts to scientist, to better describe their models, to define uncertainty and variability to refer to high quality standards for

the protocols. This will require more work, but will produce a more mature attitude, for a better collaboration between different scientists, and use of results by stakeholders.

Exploitations within EC proposals:

The debate of RAINBOW promoted collaborations in terms of proposal prepared for the 7th Framework Programme, which surely represent a better way of exploitation, since to modify description of work of contracts already financed presents some limits. The exciting discussion between partners and some of the speakers, not in the RAINBOW consortium, produced some valuable ideas, which were posed at basis of some interesting proposals for the FP7. This refers to the following proposal:

1) MOSAIC (Modular Optimised System Addressing toxicity Inducing Chemicals), for the call FP7-HEALTH-2007-A.
It is coordinated by P1 (IRFMN), with the participation of other 10 partners, such as Politecnico di Milano, Helmholtz Centre for Environmental Research - UFZ, and European Chemicals Bureau. The ideas to have a close feedback between in silico and in vitro methods has been included in MOSAIC. MOSAIC is devoted to develop in silico methods for Carcinogenicity, Mutagenicity and Reprotoxicity (CMR).

2) EMERALD (Environmental Models for Effects from stRucture-based and Alternative Decisions), for the call FP7-ENV-2007-1.
It is coordinated by Mark Cronin (Liverpool John Moores University) with the participation of other 9 partners, such as Helmholtz Centre for Environmental Research - UFZ, “Mario Negri” Institute, European Chemicals Bureau and Christina Rudén (for the Royal Institute of Technology, KTH, in Stockholm, to which she is affiliated as well). EMERALD is devoted to develop in silico methods for environmental protection. The ideas to traduce in silico results into the RA scheme has been included.

3) SENTINEL (SEcuring the food chaiN: developmenT of novel INtElLigent testing protocols to detect contaminatinghazards), for the call FP7-KBBE-2007-1.
This proposal for a large-scale Integrating Project is devoted to produce better measuring tools for food contaminants. “Mario Negri” Institute and Karolinska Institutet are involved. The idea to integrate alternative methods into a more complex schema has been included.

Deliverables List for WP 3

Del. no.	Deliverable name	WP no.	Date due	Date of preparation	Actual delivery date	Estimated indicative person-months (*)	Used indicative person-months (*)	Lead contractor
D3.1	Choice of the dissemination way of the presentations from the workshop	WP 3	Month 3 (05/2006)	Month 3 (05/2006)	Month 12 (02/2007)	0.30	0.30	P3
D3.2	Document summarising final results from the workshop	WP 3	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	0.30	0.50	P3
D3.3	The list of suitable DB and SW for RA	WP 3	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	0.40	0.70	P3
D3.4	Papers sent to journals/book	WP 3	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	1.00	1.50	P3
D3.5	Exploitation plans in other EC projects	WP 3	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	0.50	0.55	

D3.6	Summary of dissemination and exploitation tools	WP 3	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	0.50	0.50	
						3.00	**4.05**	

(*) INCLUDING, IF AVAILABLE, PERSON-MONTHS NOT COVERED BY THE RAINBOW GRANT FOR P1 (IRFMN) AND P3 (KI)

Milestones List for WP 3

M. no.	Milestone name	WP no.	Date due	Date of preparation	Actual delivery date	Lead contractor
M3.1	The dissemination through journal/web	WP 3	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	P3
M3.2	Summary of dissemination and exploitation tools	WP 3	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	P3

WP 4: The web site

Objectives

- To prepare a web site for the Project, with full details of the workshop, and after the workshop, to present main results for the workshop.

The milestone for this WP was the web site with call and topics.

Activities

A dedicated web site was created and maintained, at the address http://www.rainbow-project.eu/. There were three main moments in the life of the web page.

Before the workshop and during the organization phase, the web site was built up with the aim to give an immediate information about the workshop and the project, with the indication on the home page of details such as the dates and venue of the event, the procedure for registration and the advertisement for the nine available fellowships.
Contact details, information about the consortium, topics and objectives of the project, call for the workshop, questions to be addressed at the workshop, have been provided in specific sections.

Then, once defined, the web site listed the detailed planning of the workshop with the agenda and schedule of presentations (downloadable as PDF file), the priorities and list of speakers.

After the workshop, the web site is now and will be in the future a repository for all documents summarising results, conclusions, recommendations from the workshop, together with the list of databases and web resources for risk assessment, and an animated didactic presentation of in silico and in vitro models ("*QSAR approach to predict toxicity*", available through a link on the home page or at the address http://www.rainbow-project.eu/qsar.html).

The web site also provides references to EC funded projects (such as CAESAR and DEMETRA) and to the European Commission, with acknowledgements.

In the next pages *Figure 1* shows a screenshot of the current home page of the RAINBOW website (with the link to the animated didactic presentation on QSAR), while *Figure 2* and *3* show screenshots for the report and web resources pages.

Figure 1: the RAINBOW website - Home Page

Figure 2: the RAINBOW website - Report Page

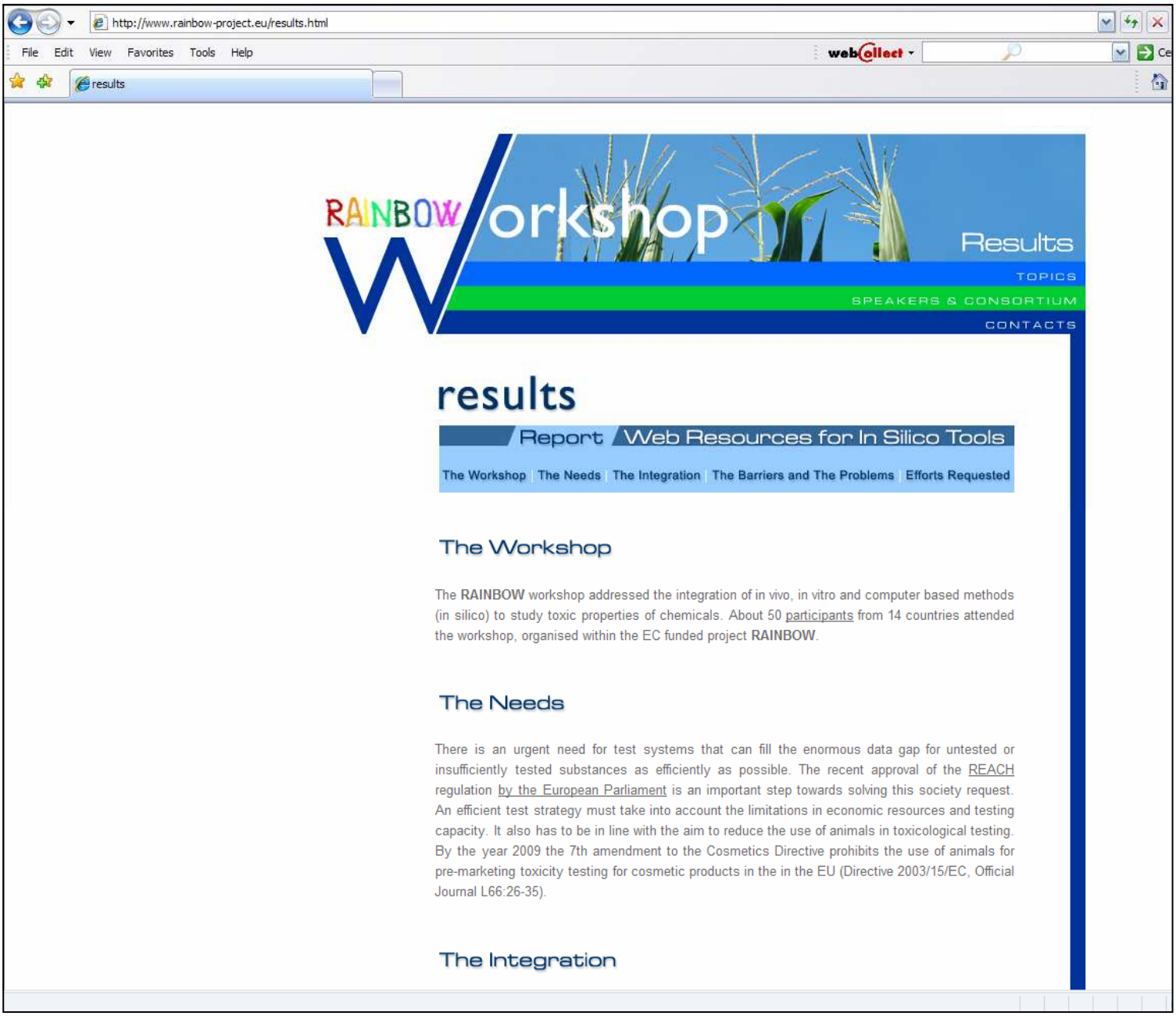
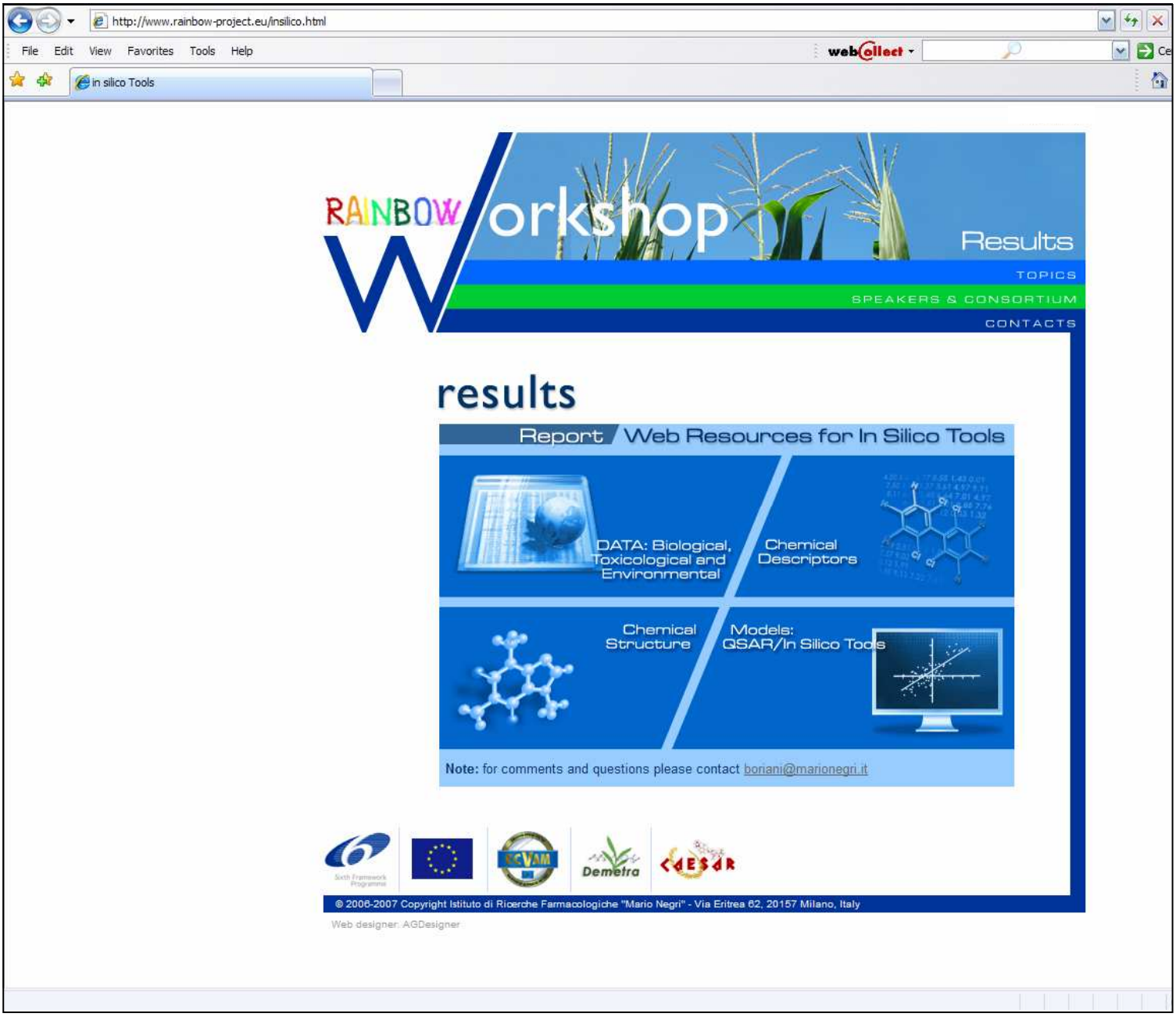


Figure 3: the RAINBOW website - Web Resources Page



Deliverables List for WP 4

Del. no.	Deliverable name	WP no.	Date due	Date of preparation	Actual delivery date	Estimated indicative person-months (*)	Used indicative person-months (*)	Lead contractor
D4.1	The web site with main information on the workshop, such as call, topics, venue, date	WP 4	Month 2 (04/2006)	Month 3 (05/2006)	Month 12 (02/2007)	0.30	0.40	P1
D4.2	The list of selected presentations and final programme of the workshop will be added	WP 4	Month 7 (09/2006)	Month 8 (10/2006)	Month 12 (02/2007)	0.10	0.10	P1
D4.3	The document with the conclusions of the workshop will be added	WP 4	Month 11 (01/2007)	Month 11 (01/2007)	Month 12 (02/2007)	0.10	0.30	P1
						0.50	**0.80**	

(*) INCLUDING, IF AVAILABLE, PERSON-MONTHS NOT COVERED BY THE RAINBOW GRANT FOR P1 (IRFMN) AND P3 (KI)

Milestones List for WP 4

M. no.	Milestone name	WP no.	Date due	Date of preparation	Actual delivery date	Lead contractor
M4.1	Web site with call and topics	WP 4	Month 2 (04/2006)	Month 3 (05/2006)	Month 12 (02/2007)	P1

WP 5: Management

Please consider *Section 3 - Consortium Management* for detailed info about the activities within this WP.

Section 3. Consortium management

All the activities in WP 5 were aimed to favour the successful organization of the workshop event and to coordinate efforts, ensuring the co-operation between the members of the consortium and the exchange of information, for the dissemination and exploitation of the workshop outcomes.

Objectives

- To manage the project in conformity with the terms of the Model Contract and the Technical Annex so as to achieve the milestones and produce the deliverables on time, according to the budget, and ensuring a high quality level.
- To make use of synergies by coordination and information exchange.
- To ensure information exchange between members of the consortium.
- To coordinate the organisation of the workshops and meeting.
- To control the correct dissemination and exploitation of the results.

The milestone for the workpackage on management was the final report with cost statements.

Activities

Synergies and information exchange between members of the consortium were assured by the constant e-mailing along the whole duration of the project, and by meetings involving the members of the Steering Committee.
In particular, the coordinator (P1) organized the kick-off meeting in Milan (16 March 2006), in order to fine-tune the project within the consortium: in that circumstance, the dates of the workshop were fixed, thematic areas were better defined, and the Steering Committee discussed about the identification of participants in the workshop and practical aspects of the organization. The minutes of the kick-off meeting were prepared and distributed within the consortium.
Further restricted meetings (with the actual participation of the coordinator, P1, with representatives of P3) was held in Milan (20 November 2006) and Brussels (5 February 2007) in order to discuss dissemination aspects.
The second meeting after the workshop was not held in an official form, on a collective decision of the Steering Committee, but substituted by restricted talks among the members of the consortium, in order to define dissemination aspects in particular. Besides, to continue and improve the discussion on inputs emerged during the workshop, contacts were assured by phone calls and e-mails in the following months.

As regards the organization of the workshop, the Local Organizing Committee (mainly composed by representatives of the coordinator, P1, and of P2, POLIMI) prepared the final programme of the workshop and concentrated on the definition of the practical details of the event.
The LOC, in order to provide an optimal accommodation, identified a single hotel (Enterprise Hotel in Milan) to host all the participants of the workshop and prepared social events (social dinner and visit to an art exhibition) after the workshop sessions.
The LOC, on behalf of the Steering Committee, contacted speakers interested to present their contributions and defined the schedule of presentations on the basis of themes and arguments.
The LOC invited and contacted potential participants and ensured them a constant and prompt assistance before and during the workshop, supplying details about the workshop event and venue,

and providing practical (bags, pens, block-notes, etc.) and informative (schedule of presentation and discussions) materials.

Concerning dissemination aspects, these were ensured during the preparation of the workshop through the web site of the project (built up with the help of a web designer as subcontractor for the graphical part and for animations), which has been updated time by time as described in WP 4, and through advertising circulated via e-mail by the coordinator (P1).
The workshop event has been publicized also through the participation of members of the consortium to meetings and conferences (see WP 1).
After the workshop, the dissemination activities included a summary of the discussion at the workshop, with main results, a list of database and SWOT analysis for Risk Assessment, submitted papers, including one which summarize the project results and that will be published on ATLA (with contributions by both members of the Steering Committee and participants of the workshop), and the final update of the web site (the web site was updated with the report of the project, http://www.rainbow-project.eu/results.html, and a list of suitable database and software for in silico tools was created and put available on line at the address http://www.rainbow-project.eu/insilico.html).
Inputs and ideas from the workshop will continue to be discussed and improved within the activities of some proposed projects for the 7th Framework Programme (i.e. MOSAIC, coordinated by P1, EMERALD, SENTINEL), if approved.

Deliverables List for WP 5

Del. no.	Deliverable name	WP no.	Date due	Date of preparation	Actual delivery date	Estimated indicative person-months (*)	Used indicative person-months (*)	Lead contractor
D5.1	Kick-off meeting	WP 5	Month 1 (03/2006)	Month 01 (03/2006)	Month 12 (02/2007)	1.00	1.20	P1
D5.2	Second meeting	WP 5	Month 9 (11/2006)	Month 10 (12/2006)	Month 12 (02/2007)	1.00	0.60	P1
D5.3	The final report with cost statements	WP 5	Month 12 (02/2007)	Month 12 (02/2007)	Month 12 (02/2007)	1.00	1.30	P1
						3.00	**3.10**	

(*) INCLUDING, IF AVAILABLE, PERSON-MONTHS NOT COVERED BY THE RAINBOW GRANT FOR P1 (IRFMN) AND P3 (KI)

Milestones List for WP 5

M. no.	Milestone name	WP no.	Date due	Date of preparation	Actual delivery date	Lead contractor
M5.1	Web site with call and topics	WP 5	Month 12 (02/2007)	Month 12 (02/2007)	Month 12 (02/2007)	P1

Contractors: Comments regarding contributions, changes in responsibilities and changes to consortium itself, if any

In the very last period of the project, after the Workshop, Dr Wilhelmina Hermans left P5 (IVTIP). This caused a gap in the communication with IVTIP, due to the fact that Dr Hermans was the main actor and contact person of RAINBOW for the IVTIP.

This caused also unexpected delays in the transmission of documents and contributions (both for activity and management information) to the coordinator from Partner 5 (IVTIP), and consequently in the submission of the final reports to the European Commission.

Only after multiple solicitations by the coordinator to Partner 5 it was possible to collect the information in the activity report and, as regards the management section, consolidated expenses by Partner 5 have been notified to the coordinator only on 24 May 2007.