OSIRIS Newsletter

No. 5 November 2009

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Photo: Andrea Richarz

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Third OSIRIS Annual Meeting

Photos: Mark Hewitt

The Third OSIRIS Annual Meeting will take place

on 9 – 11 March 2010
in Liverpool, UK.

It will be hosted by the OSIRIS partner Liverpool John Moores University.

The meeting venue is the Holiday Inn Hotel, Lime Street, located centrally in Liverpool City Centre.

On the agenda:

- Results of the 3rd project year
- Planning for the next months
- Intra- and inter-Pillar/-Workpackage discussions.

The OSIRIS conference dinner will be held at the historic Liverpool Athenaeum Club, a unique opportunity to see inside a traditional English "Gentleman's Club" which holds Liverpool's first library.

OSIRIS Partners

Universiteit Antwerpen – UA (Partner 12)

Laboratory of Ecophysiology, Biochemistry & Toxicology
Antwerp, Belgium

Deputy Cross-cutting Co-ordinator of Experimental Work
WP 2.3 leader

Photo: : © Hugo Maertens

The Laboratory for Ecophysiology, Biochemistry and Toxicology (EBT) is part of the Department of Biology of the University of Antwerp (UA), Belgium, consisting of several research units with in total about 150 staff members and Ph.D. students.

The laboratory facilities include different types of exposure systems and holding tanks to perform both short and long-term exposure experiments with aquatic species, eukaryotic cell culture facilities and a molecular biology lab.

EBT Research Focus:

The EBT research focuses on the molecular and biochemical aspects of microcontaminants (metals as well as organics) and the physiological aspects of adaptation to environmental stress.

Within OSIRIS, the possible contribution of a transcriptomics approach to the qualitative/quantitative structure-activity relationship (QSAR) methodology is investigated, evaluating whether biological ("omics") data can support a mode of action (MOA) classification in order to improve the accuracy of the QSAR estimates for environmental hazard endpoints. Toxicogenomics, combined with physiological parameters, are applied for elucidating MOA of different toxicants according to the Verhaar classification scheme. Daphnids and algae are being used as test organisms.

Stockholm University – SU (Partner 21)

Department of Applied Environmental Science (ITM)
Unit for Analytical Environmental Chemistry
Stockholm, Sweden

Photo: ITM

The Department of Applied Environmental Science (ITM) at Stockholm University consists of approximately 135 persons. ITM undertakes private and public funded research in the following disciplines: atmospheric chemistry and physics, biogeochemistry, environmental organic and inorganic chemistry, limnology, ecology and ecotoxicology, whereby the majority of the activities are linked to trace organic contaminants.

Unit for Analytical Environmental Chemistry:

The research is focused on the development of analytical methods to measure organic contaminants in the environment, and on the application of these methods to further understanding of contaminant sources, transport, fate, bioaccumulation, and effects.

Within OSIRIS, SU-ITM develops improved modelling tools to assess exposure to chemicals and will give recommendations for the update of the EU Technical Guidance Documents TGD and the tools for the Chemical Safety Assessment (e.g. EUSES). The work focuses on bioaccumulation modelling. Among the planned innovations are the introduction of polyparameter linear free energy relations (ppLFERs) into the description of phase partitioning in the models, and the expansion of their range of application to polar chemicals. The activities build on the ACC-HUMAN model that has been developed by the ITM group.

OSIRIS Partners

Centre National de la Recherche Scientific – CNRS (Partner 24)

Laboratoire Ampère
Environmental Microbial Genomics Group
Lyon, France

The CNRS, "Centre National de la Recherche Scientific", France, is a public organisation for scientific and technological research and is under the authority of the French Ministry for Research.

The Environmental Microbial Genomics Group:

The Environmental Microbial Genomics Group within the Laboratoire Ampère (UMR CNRS 5005) includes researchers and professors from both the CNRS and the Ecole Centrale de Lyon. The current research projects are funded by the EU and several national and regional agencies.

In general, the group works in different areas related to the OSIRIS project including environmental engineering, biodegradation, environmental microbiology, bacterial adaptation to chlorinated compounds and heavy metals, gene transfer between bacteria, pollutant degradative pathways and kinetics.

Photo: Timothy Vogel

In addition, microbial activity in different environments such as soils related to both general respiration rates, gene transfer and bioavailability are current themes of research.

Within OSIRIS, the group addresses experimental biodegradation in soil as measured by oxygen consumed per kilogramme of soil, studying different confounding factors, in order to provide input for model development and validation.

Analytical Laboratory Luhnstedt – AL (Partner 28)

Luhnstedt, Germany

WP 1.1 leader

Analytical Laboratory Luhnstedt (AL), Germany, is a private institute (SME) for analytical chemistry, environmental data analyses and ecotoxicological modelling. AL provides applied research, e.g. studies and research projects for the EU, OECD, UBA (German Environmental Agency), DMU (Danish Environmental Agency) and US EPA. Emphasis is laid on integrated approaches combining environmental chemistry and (eco)toxicology.

AL features a fully equipped laboratory (accredited according to DIN ISO/IEC 17025) for performing physicochemical measurements and analyses of organic and inorganic compounds in various media.

AL Research Focus:

AL research addresses aquatic toxicity, bioaccumulation and persistence of chemicals in limnic and marine ecosystems. The methods and competences comprise quantitative structure-activity relationships

Photo: AL-Luhnstedt

(QSARs) with emphasis on principal (rate-limiting) processes. Multivariate (eco)toxicity profiles have been developed for the analysis and classification of contaminants by mode(s) of action (MOA). These have been linked with interspecies correlations and *in vitro* methods as tools for replacing animal testing.

Hazard and risk assessment methodology has been applied in PBT-assessment, to identify potential SVHC candidates and to derive water quality criteria (suggested) for German Competent Authorities (UBA, LAWA).

OSIRIS Partners

Cyprotex Discovery Ltd. – CYPROTEX (Partner 30)

Scientific Computing Group
Macclesfield, United Kingdom

WP 3.3 leader

Cyprotex is an SME, currently with 45 members of staff. It is a specialist provider to industry of tools and services related to the determination and prediction of absorption, distribution, metabolism, elimination, toxicity and pharmacokinetics/toxicokinetics (ADMET/PK). It provides off-the-shelf and bespoke *in silico* solutions for predicting ADMET/PK, as well as *in vitro* screening of ADMET and physicochemical properties.

Cyprotex's facilities comprise a unique combination of robotic liquid-handling hardware, in-house laboratory systems, databases and automated PBPK and QSPR model development. These facilities enable reproducible, rapid-turnaround, high-availability data generation, and rapid, incremental model generation and evaluation. Well organised data abstraction processes, backed by bespoke automation anddatabases, facilitate low error data curation from external sources to further support model development.

Photo: © Cyprotex

Cyprotex Scientific Computing Group:

The Scientific Computing Group have many years experience in the development and application of physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) simulation, and quantitative structure/property relationship (QSPR) models, and have developed a unique, generic PBPK model (Cloe® PK), for prediction of mammalian xenobiotic kinetics following administration via intravenous and oral routes.

University of Wageningen – WUR (Partner 31)

Department of Social Sciences
Environmental Economics and Natural Resources Group
Wageningen, The Netherlands

The Wageningen University and Research Center (WUR) comprises leading research institutions in the Netherlands, covering all aspects related to agriculture and the up- and downstream sector. WUR activities focus on ensuring reliable supplies of safe, high-quality food while maintaining the biodiversity of natural habitats and conserving natural resources.

Photo: University of Wageningen

The Environmental Economics Group:

Research of the Environmental Economics Group focuses on the economic analysis of national and international environmental problems, in order to contribute to a better understanding, identify possible solutions, and give policy recommendations. It aims at integrating insights from natural sciences and technology with economic expertise, with emphasis on information aggregation needed for assessing impacts, cost-effectiveness and efficiency of policy options. Applications of modelling approaches include computable general equilibrium models (CGE), bioeconomic and spatial as well as game theoretic models. Theoretical and methodological research is carried out in the areas of environmental valuation, productivity and efficiency analysis, and institutional economics, including the economics of climate change, general equilibrium modelling of energy, pollution and waste, economics of resources and biodiversity, water management, and biotechnology.

OSIRIS Partners

DIALOGIK – DIA (Partner 29)

Department of Communication, Perception and Participation
Stuttgart, Germany

DIALOGIK is a non-profit institute conducting systematic research into communication processes and interactions between politics, economy and civil society. It develops, implements and evaluates innovative forms of communication and new methods of participation and cooperation, in order to improve the governance of valuable resources, be they natural, economic or social.

The analyses are carried out at the local, regional, national and transnational level. The research team has special expertise in social scientific approaches.

Department of Communication, Perception and Participation:

The Department of Communication, Perception and Participation investigates processes of public dialogue, social interaction and decision making in politics, economy and society. It studies the perception and acceptance of risk and technologies, develops tailor-made communication programmes, conducts participation projects and organises stakeholder and public involvement processes.

Photo: DIALOGIK

For empirical analyses methods such as expert delphis, group delphis, focus groups and open space conferences are used; procedures such as round tables, consensus conferences, citizen panels or juries, future workshops and mediation are applied for participatory purposes.

New OSIRIS Publications

The complete publication list with **links to the articles** is available at http://www.osiris-reach.eu

Publications in Peer Reviewed Scientific Journals

- Enoch SJ, Madden JC, Cronin MTD 2008. Identification of mechanisms of toxic action for skin sensitisation using a SMARTS pattern based approach. SAR QSAR Environ. Res. 19 (5-6): 555-578
- Madden JC, Enoch SJ, Hewitt M, Cronin MTD 2009. Pharmaceuticals in the environment: Good practice in predicting acute ecotoxicological effects. Toxicol. Lett. 185 (2): 85-101
- Schwöbel J, Ebert R-U, Kühne R, Schüürmann G 2009. Prediction of the intrinsic hydrogen bond acceptor strength of chemical substances from molecular structure. J. Phys. Chem. A. 113 (37): 10104–10112
- Hewitt M, Cronin MTD, Enoch SJ, Madden JC, Roberts DW, Dearden JC 2009. *In silico* prediction of aqueous solubility: the solubility challenge. J. Chem. Inf. Model. 49 (11): 2572–2587
- Fjodorova N, Vračko M, Tušar M, Jezierska A, Novič M, Kühne R, Schüürmann G 2009. Quantitative and qualitative models for carcinogenicity prediction for non-congeneric chemicals using CP ANN method for regulatory uses. Mol. Divers., in press, available online
- Kahru A, Dubourguier HC 2009. From ecotoxicology to nanoecotoxicology. Toxicology, in press, available online
- Kühne R, Ebert R-U, Schüürmann G 2009. Chemical domain of QSAR models from atom-centered fragments. J. Chem. Inf. Model., in press, available online
- Smith KEC, Oostingh GJ, Mayer P 2009. Passive dosing for producing defined and constant exposure of hydrophobic organic compounds during in vitro toxicity tests. Chem. Res. Toxicol., in press, available online

Second OSIRIS Training Course

Objectif

The aim of the Second OSIRIS Training Course was to provide an introduction to the main concepts underlying the design of Integrated Testing Strategies (ITS) for the regulatory purposes of REACH and to provide some practical experience of the application of various software tools relevant to ITS. The course was aimed at **professional end-users in industry** and **regulatory agencies** as well as at **young scientists**.

The course was held on **23–25 September 2009** at the **Mario Negri Institute in Milan**, Italy, co-chaired by Emilio Benfenati (IRFMN) and Andrew Worth (JRC). It was divided into two modules – Module I was a **theoretical introduction to risk assessment** and the concepts underlying various software tools, whereas Module II was devoted to **software demonstrations** and hands-on case studies.

Module I

In Module I, Kees van Leeuwen (TNO) gave an interactive introduction to the role of risk assessment in the control of chemicals and its relation with risk management. He also reported on REACH and the need for ITS, describing the underlying principles and difficulties encountered.

Yuri Bruinen de Bruin (RIVM) explained the REACH procedures and the Chemical Safety Assessment, with a focus on exposure assessment.

Silke Gabbert (WUR) gave and introduction to Cost-effectiveness Analysis (CEA), illustrating the use of CEA for the assessment of the performance of chemical tests and testing strategies and discussing the application as a decision-support tool.

Alessandra Roncaglioni (IRFMN) informed about bioaccumulation and the endpoint bioconcentration factor, important for REACH within the context of PBT assessment and classification and labelling.

Romualdo Benigni (ISS) described the development of strategies for *in vitro*, *in vivo* and *in silico* information for mutagenicity/carcinogenicity.

Mark Cronin (LJMU) reported on the use of information relating to mode and mechanism of toxic action in ITS for toxicity prediction and outlined different techniques to determine mechanisms of action.

Andrew Worth gave a presentation on the reporting formats used to document QSAR models and their predictions.

Module II

In Module II, demonstrations and practical sessions were provided of Toxtree by Arianna Bassan (Soluzioni Informatiche; S-In), of the OECD QSAR Toolbox by Mark Cronin, of CAESAR models by Alessandra Roncaglioni, of the ChemProp software by Andrea Richarz (UFZ), and of the OSIRIS Web Tool by Eduard Pauné (SIMPPLE).

As part of Module II, there was also an open discussion on the **adequacy of QSARs** and what steps are still needed to **promote the acceptance** of the models. It was suggested that there should be an EU-certified course on computational toxicology which would ensure a minimal qualification and level of assurance for laboratories/consultants involved in providing QSAR data for REACH registration dossiers.

Photos: Andrea Richarz

Further information

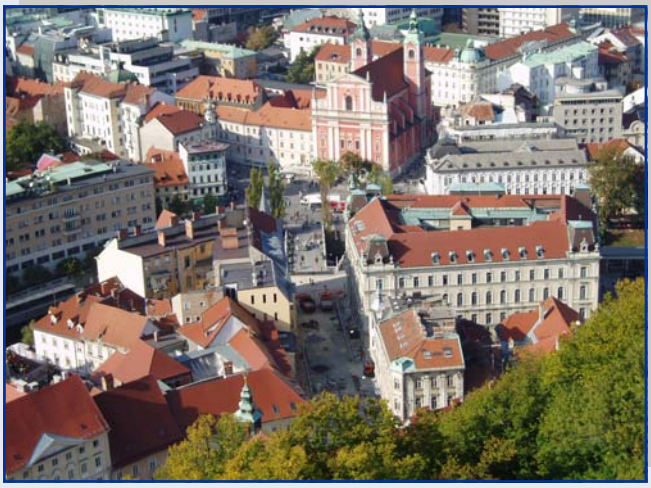
The detailed programme and presentation abstracts are available at the project website:

http://www.osiris-reach.eu.

Pillar 2 Meeting in Ljubljana

The OSIRIS Pillar 2 meeting was held at the **Institute of Public Health in Ljubljana**, Slovenia from **8–9 October 2009**. The primary purpose of the meeting was to discuss results, and especially to allow the younger researchers to present their findings in a friendly environment. All together 24 participants were present representing 11 partners of the OSIRIS project.

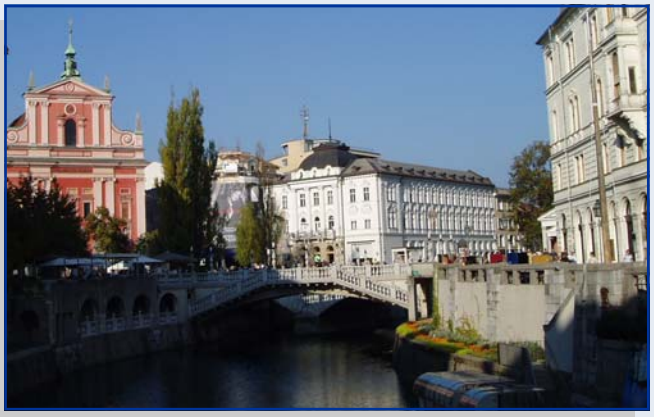
The meeting started with the conclusions from the **Liverpool Mode/Mechanism of action Workshop**. Several studies were presented where chlorinated anilines were used as case study substances. Transcriptional responses of *Folsomia candida* were studied by exposure to a series of **chlorinated anilines** and showed no relationship to log K_{ow} or with increasing chlorine substitution. Toxicity of chlorinated anilines to algae and luminescent bacteria was presented. Quantitative Activity-Activity Relationship (**QAAR**) and Quantitative Structure-Activity Relationship (**QSAR**) **approaches** with a new complete set of original experimental data were discussed. The validation of a QSAR approach using multiple species was also presented for chlorinated anilines. Furthermore the progress in development of QSAR models for soil toxicity by performing standard toxicity tests with the soil dwelling springtail *Folsomia candida* (Collembola) was presented. One of the conclusions was that *Folsomia candida* is more sensitive to chloroanilines and chlorobenzenes than other soil organisms. A plan of how to use *in vitro* assays to predict *in vivo* responses with help of physiologically based pharmacokinetic (**PBPK**) **modelling** and QSARs for protein binding was presented. The participants also discussed **passive dosing** when used in the Ames II genotoxicity test and when *in vitro* and *in vivo* toxicity results using passive dosing



were compared to chemical activity. An across species comparative toxicity study of organic compounds was discussed and several issues relating to **data quality and variability** were raised. Optimisation of *in vivo* testing procedures also related to the same questions. **Intelligent testing strategies** were proposed for optimisation of aquatic fish toxicity assessment and reduction of vertebrate use as well as the application of available and relevant **read-across methods** to case study compounds for drinking water. Finally, the ChemProp chemical properties estimation software system was presented.

The participants found the presentations and the discussions good and useful. Several times they have emphasised the need for greater numbers of good quality data which would allow a better extrapolation of conclusions to the REACH chemicals.

Intense discussions continued well into the evening in the lively atmosphere of the city centre. The need for more similar meetings was the final conclusion of all participants.



Photos: Andrea Richarz

Pillar 4 Meeting in Hannover

On **4–5 November 2009**, the participants of the various workpackages within Pillar 4 (Integration Strategies and Tools), i.e. WP 4.1 Regulatory domain and factors affecting acceptance, WP 4.2 Framework for human health endpoints, WP 4.3 Framework for environmental endpoints, WP 4.4 Decision theory and tools, met to discuss the progress of the Integrated Testing Strategies (ITSs) which are being developed. The meeting was hosted by the **Fraunhofer Institute of Toxicology and Experimental Medicine** (ITEM) **in Hannover,** Germany.

Lively discussions were held about the **underlying ITS concepts**, decision theory, cost-effectiveness analysis, the practical **ITS tools** and the OSIRIS web tool. The purpose of the meeting was to share information and concepts, but also to set a path to 'handing over' (preliminary) ITSs to the first users in Pillar 5 (Case Studies). This step is considered essential, since it provides the developers with **feedback** which they can use to improve their ITSs and guidance documents.

Photo: Pillar 4

Photo: Fraunhofer-Gesellschaft

The following ITSs were considered to be ready for testing by Pillar 5 by end of this year or early 2010:

Human Health:
- mutagenicity
- skin sensitisation

Environment:
- bioconcentration factor
- aquatic toxicity
- possibly biodegradation.

In addition, work is ongoing on an ITS for repeated dose toxicity, which will be the proof of principle for a continuous endpoint for Human Health. While this is clearly more complex than the categorical endpoints, good progress is being made.

OSIRIS at the VII World Congress on Alternatives & Animal Use in the Life Sciences

The 7th World Congress on Alternatives & Animal Use in the Life Sciences took place from **30 August to 3 September 2009 in Rome**, Italy, in the year of the 50th anniversary of Russell & Burch's **3Rs principle** reduction, refinement and replacement.

OSIRIS was strongly represented in two sessions, chaired by the Co-ordinator Gerrit Schüürmann.

Session „Integrated Approaches"

The goal of the session was to review the development, scope and application – as far as available – of major integrated testing strategy (ITS) components from the perspective of an integrated approach for chemical safety evaluation, taking into account the 3Rs principle.

Gerrit Schüürmann, UFZ, summarised the concept of ITS for chemical safety assessment. ITS consider all available information including non-test information such as qualitative and quantitative structure-activity relationships (QSARs), chemical and biological read-across, data from *in vitro* tests, chemo-assays and -omics, as well as exposure analysis and thresholds of toxicological concern (TTC), building on a weight of evidence consideration of uncertainties to derive adequate conclusions for the specific question.

Theo Vermeire, RIVM, described criteria for exposure based waiving (EBW) as foreseen in the REACH regulation. EBW applies in situations where human or environmental exposure is so low that there is a very low probability for additional effect information to improve the ability to manage risk. EBW justification can be qualitative or quantitative, the latter based on the exposure scenario developed in the REACH Chemical Safety Report and the models recommended in the ECHA Technical Guidance.

Ovanes Mekenyan, LMC, detailed the chemical category evaluation approach. Closely related chemicals are considered as a group – chemical category – rather than as individual chemicals. In this way, available test data can be used to estimate the corresponding properties for untested chemicals and endpoints instead of testing everything. This data gap filling may be achieved through read-across, trend analysis or (Q)SAR models.

Inge Mangelsdorf, FhG, presented a study on the applicability of TTC to industrial chemicals under REACH, in the context of EBW. The TTC concept describes thresholds below which no appreciable risk to human health is assumed. The study focused on repeated dose toxicity studies with rodents for existing chemicals, using the database RepDose. The data showed that TTC are applicable for industrial chemicals, yet further refinements are desirable.

Dinant Kroese, TNO, described the weight-of-evidence (WoE) approach for combining testing and non-testing information in ITS under development in OSIRIS. It is based on Bayesian networks and will provide the criteria the available information has to meet in order to be considered adequate for classification & labelling or risk assessment.

Session „Status report on OSIRIS“

Gerrit Schüürmann, UFZ, gave a general overview of the project and the recent developments.

Helmut Segner, UB, discussed the possible role of *in vitro* approaches – not yet used in regulatory ecotoxicological testing – for ecotoxicological hazard assessment. The current focus is on the substitution of vertebrate *in vivo* tests. Another possible role is to reduce uncertainties. *In vitro* assays, screening for specific toxic reactivities and modes of action, can provide “alerts” helping to prioritise and guide subsequent testing, and may be useful in acute-chronic as well as in across-species extrapolations.

Romualdo Benigni, ISS, reported on integration strategies for mutagenicity/carcinogenicity. *In vivo* studies for these endpoints pose a high demand for test-related resources. Therefore, the development and use of *in silico* estimation techniques might have a huge saving potential. Uncertainties are a crucial issue, they are found in both modelling and experimental methods. Thus both method types should be exploited adequately and combined.

Joanna Jaworska, P&G, described a developed quantitative WoE framework for evaluation of non-animal information to support decision making in the form of a Bayesian Network. It consistently evaluates existing information, resolves conflicting evidence, interprets multiple test battery results including reduction in the battery outcome certainty due to conditional dependence between tests. The framework was applied to assess skin sensitisation based on three lines of evidence: bioavailability, peptide reactivity and dendritic cell activation.

Third OSIRIS Stakeholder Workshop

The Third OSIRIS Stakeholder Workshop will be held on **1–2 March 2010**, organised by the OSIRIS partner DIALOGIK. It is aimed at continuing the dialogue between the project and EU stakeholders.

The OSIRIS concepts and applications of the Integrated Testing Strategies (ITS) developed will be presented, the **OSIRIS web tool for ITS** and its functionalities will be demonstrated and **case studies** will be discussed.

Case studies planned:

- Bioconcentration factor (BCF)
- Skin sensitisation.

Stakeholders from industry, regulatory authorities, NGO’s and academia are invited to provide valuable input to the OSIRIS team, to bring forward concerns and expectations and to discuss applications of the OSIRIS research in the REACH risk assessment process.

Details on the programme and registration will be published on the OSIRIS website **www.osiris-reach.eu**. For pre-registration and notification via email, please send an email to **osiris@ufz.de**.

OSIRIS Results Highlights

Optimisation of *in vivo* testing strategies – Implementing the 3Rs (Partners LJMU, RIVM, TNO)

School of Pharmacy and Chemistry, Liverpool John Moores University, UK
RIVM, Laboratory for Health Protection Research, Bilthoven, The Netherlands
TNO Quality of Life, Zeist, The Netherlands

Development of tools to predict toxicity, within the OSIRIS project, is directed towards the minimisation of animal testing through the **integrated use of alternatives**, such as computational modelling and *in vitro* testing. Where animal testing is deemed necessary, i.e. the information is not available and could not be obtained by other means, implementation of the **3Rs strategy (reduction, refinement and replacement)** is essential.

The first of two project reports on optimisation of *in vivo* testing strategies related to the **identification of key elements for optimisation** in *in vivo* testing strategies.

A **workflow** was produced to serve as guidance when the use of *in vivo* assays is being considered. This identified aspects such as: searching for existing data, compound selection, choice of the least severe techniques, use of appropriate statistical methodologies to ensure adequate power of experiments, adequate staff training, complete documentation of process and justification of methods etc.

Additionally, four endpoints were selected (**skin sensitisation, reproductive toxicity, mutagenicity and carcinogenicity**) and methods employed to determine these toxicities were reviewed. For each of these endpoints both generic and endpoint-specific sources of variability in experimental procedures were reported. **Generic sources of variability** (i.e. those which may arise in many assays) include factors such as vehicle or control group selection, housing, genetic make-up of test species etc. **Endpoint-specific variation** includes factors only relevant for a given endpoint, for example the use of occluded or non-occluded systems in skin sensitisation testing. Tables identifying such sources of variability have been generated. Minimising this variability is one potential area where *in vivo* testing could be optimised.

These aspects and other opportunities to optimise *in vivo* testing will be explored further. It is anticipated that this work will provide more guidance in practical ways to implement the 3Rs strategy, for example, using combined tests to reduce the overall number of animals used, replacing higher species of animals with lower species and better use of statistical methods in experimental design.

We would welcome other views and perspectives on this topic. Hence, if anyone has comments on optimisation of *in vivo* testing or would like to recommend additional material relevant to the discussion, please contact Judy Madden (j.madden@ljmu.ac.uk).

Optimised experimental methods to determine biodegradation rates (Partners ECT, CNRS)

ECT Oekotoxikologie GmbH, Flörsheim/Main, Germany
CNRS, Environmental Microbial Genomics Group, Laboratoire Ampère, Lyon, France

Due to the heterogeneity and complexity of terrestrial matrices limited knowledge about the **biodegradation potential of micro-organisms** is available to predict the fate of compounds in soil.

The degradation of selected organic test compounds from various chemical classes as well as mixtures of these test substances with sodium benzoate was investigated according to a well-defined test design using soils from France (e.g. La Côte St André soil) and Germany (e.g. Lufa Standard Soil Type 2.3). For this purpose, the OxiTop® system (WTW, Germany), a rapid and efficient method recommended to

OSIRIS Results Highlights

measure soil respiration according to ISO 16072, was applied. This system determines manometric changes that occur when oxygen is consumed to transform organic carbon into CO_2. In the closed system, CO_2 is trapped by an absorbent, resulting in a pressure reduction, which is measured automatically at constant intervals und used for calculating the biological oxygen demand (BOD).

Furthermore, the influence on **soil moisture** was investigated using soda lime pellets, NaOH powder and NaOH solution as CO_2-absorber. No desiccation was observed with NaOH and only a slight decrease in soil moisture with soda lime pellets, whereas the soil was desiccated by NaOH powder.

In addition, the influence of **liquid fertiliser** and two **types of micro-organism** solutions normally used for bioremediation was investigated. No clear differences between the degradation curves using different combinations of the reference compound sodium benzoate, fertiliser and micro-organisms was observed. Slightly higher degradation rates were obtained when nitrogen-containing fertiliser was added to the soil. This is supposed to be caused by undesirable nitrification processes.

Other mixtures were tested in order to evaluate the potential induction of enzymes by one compound leading to the degradation of another compound. Moreover, the effects of different additives were examined to assess whether the measured respiration rate was limited by the **diffusion of air into the soil** in the test vessels.

The experiences and the data gained from the experiments so far showed that the soil test system can be considered as ready for use and is suitable for the generation of biodegradation data and kinetics within OSIRIS. The results showed that the biodegradation is partially dependent on the soil used and the test conditions applied. However, the variation due to different operating conditions is negligible as long as there are sufficient nutrients present for the degradation. The soil type has the most significant effect on nutrient availability.

Uncertainty and sensitivity analysis of consumer and worker exposure models

(Partners TNO, RIVM) TNO Quality of Life, Zeist, The Netherlands
RIVM, Centre for Substances and Integrated Risk Assessment, Bilthoven, The Netherlands

An important issue in evaluating the justification of **Exposure Based Waiving** (EBW) and improving the models used is the uncertainty in the estimates as well as the models' sensitivity to input changes. Examples of probabilistic assessments with direct human exposure assessment models were studied.

Worker exposure models

Two examples related to the RISKOFDERM model for dermal exposure by product distribution by hand-held tools (e.g. brushing and rolling).

- **Probability density functions** for several key determinants were used. Substantial differences in distributions resulted from different probability functions based on more general or more specific data sets, and even in the case of very specific data sets based on one scenario.
- Second, a hypothetical situation was studied with distribution of only small amounts with relatively limited variation in use rate and duration. It was shown that in specific cases, namely very specific input distributions and situations where the largest weight of all input distributions is towards inputs leading to low exposure estimates, the probabilistic exposure assessment gives output distributions and reasonable worst case values, more suitable to justify EBW than the deterministic use with one reasonable worst case input per parameter.
- A third example on exposure of nurses to anti-neoplastic agents illustrated how models based on measured relations between exposure and input parameters can be used in a probabilistic way to study the uncertainty in long term (week-based) exposure estimates.

Consumer exposure models

- The integrated dermal exposure of the adult population to musk xylene from several cosmetics products was calculated. Population variability and product uncertainty, such as product use and percentage of brands containing musk xylene, were taken into account. The importance of decreasing the uncertainty in the input parameters was illustrated.

OSIRIS Results Highlights

- Probabilistic assessments with the ConsExpo vapour model, a relatively simple consumer exposure model for exposure to vapours, and a more complex model for the same purpose, the diffusion model, were compared. Given the uncertainty in both models, it was not possible to distinguish the performance of the two models, probably due to the uncertainty in the input parameters of the more complex model. Thus the advantage of a scientifically more rigorous and complex model should be carefully weighed against the requirements of such a model regarding the input parameters.

In conclusion, the results obtained show that a justification of EBW based on generic probability density functions of the model parameters is difficult, due to the **large variability and uncertainty** in both the models and the input parameters. However, in specific cases EBW can be justified if suitable specific input data are available.

Prediction performance of QSAR models (Partner UFZ)

Helmholtz Centre for Environmental Research—UFZ, Dep. of Ecological Chemistry, Leipzig, Germany

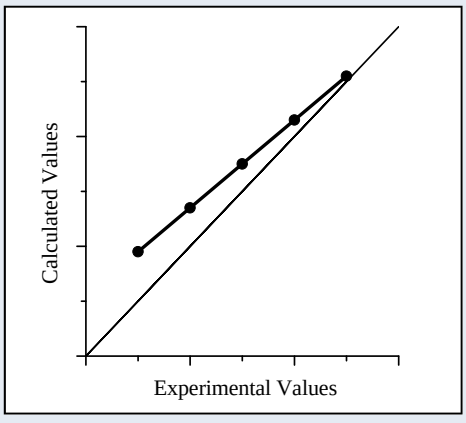
Qualitative and quantitative structure-activity relationships (QSARs) are important tools in the context of REACH, inferring the target property or activity from molecular structure information. To competently apply QSAR methods, knowledge on the respective model reliability is essential.

Statistical means used in the **quantitative evaluation** of **QSAR model performance** have been critically examined and improved.

The conventional squared correlation coefficient, r^2, calculated to measure the external prediction capability of QSAR models, implicitly applies a post-processing of the model output in terms of least-squares rescaling of the predicted values. This is not the case for the **predictive squared correlation coefficient**, q^2, which relates the predictive residual sum of squares, PRESS, to the activity sum of squares, SS. q^2 compares the untuned output of the original model to the experimental data (see figure).

In both literature and in the current OECD guidelines it is proposed to calculate q^2 with SS referring to the training set activity mean. A detailed analysis has shown that the recommended mathematical formula differs from this proposed one: Through both a mathematical proof and test calculations it was demonstrated that when employing q^2 for external validation, the associated sum of squares SS should refer to the test set under investigation rather than to the training set used for the original model derivation. The study showed that the difference between the training and test set activity means causes a **systematic overestimation** of the prediction capability in the **OECD guideline** approach for q^2. Example calculations further illustrated that for external test sets q^2 based on the training set activity mean may become even larger than r^2.

To avoid these previously unknown pitfalls, it is suggested to correct the mathematical definition of the predictive squared correlation coefficient and to always use the **test set activity mean** for quantification of the external prediction capability. The OECD guidance should be revised accordingly.



The five points perfectly fit to a line, r^2 is 1.0. However, the slope of this line is not 1, and there is an intercept different from 0. The calculated values do not correctly reflect the experimental data and thus yield an q^2 below 1.

Additional information: Schüürmann G, Ebert R-U, Chen J, Wang B, Kühne R 2008. External validation and prediction employing the predictive squared correlation coefficient - test set activity mean vs. training set activity mean. J. Chem. Inf. Model. 48: 2140-2145

OSIRIS Results Highlights

Assessment of acute/chronic relationships in aquatic toxicity (Partners UB, IVZRS)

Centre for Fish and Wildlife Health, University of Bern, Switzerland
Institute of Public Health of the Republic of Slovenia, Centre for Environ. Health, Ljubljana, Slovenia

Chronic or long-term toxicity testing aims to determine whether prolonged exposure to lower concentrations of chemicals will have significant adverse effects on biota. In the EU regulation REACH, information on chronic toxicity in the dossier is a key parameter for derivation of predicted no effect concentration (**PNEC**) values as well as for PBT (persistent, bioaccumulative, toxic) assessment for certain substances. A **PBT/vPvB assessment** is required for all substances for which a chemical safety assessment (CSA) must be conducted. These are in general — with defined exemptions — all substances manufactured or imported in amounts of 10 or more tonnes per year. In the first step of the PBT/vPvB assessment, the available information on intrinsic properties of a substance has to be compared with the criteria for persistency, bioaccumulation and toxicity. These criteria are set out in Annex XIII.

Toxicity (T) classification for the aquatic environment is based primarily on chronic no effect concentration (NOEC) values. Only if chronic toxicity data are not available, screening criteria may be used as surrogate information, and the question whether a chronic toxicity test is still required depends on the outcome of the screening process.

Research in OSIRIS examined, using aquatic toxicity as an example, whether **extrapolation from acute toxicity data** could provide a reasonable initial estimate of chronic toxicity. An evaluation of the ratio between acute and chronic toxicity values was done for fish, comparing acute lethality versus long-term toxic effects on growth or reproduction, and for daphnids, comparing acute toxicity versus chronic reproductive toxicity. In both cases, the acute-chronic ratio of the vast majority of chemicals was below 100. This ratio appears to result in less than 10% false predictions, at least for the chronic endpoints considered in this study. Thus, from a practical point of view an **acute-to-chronic ratio** (ACR) **of 100** may be used as a first estimate of the long-term toxicity of chemicals which lack chronic toxicity data. Such a screening approach is of particular value since reliable *in silico*, *in vitro* or short-term *in vivo* methods for predicting chronic toxicity are not yet available.

Caveats in using empirical acute-chronic ratios, however, must not be overlooked:

- There exist no criteria to recognise those chemicals that are outside an ACR of 100.
- The knowledge on the application domains of such an ACR approach, both in terms of chemical groups and of chronic toxicity endpoints, is insufficient.

In conclusion, an acute-chronic ratio of 100 may serve as **part of an integrated weight-of-evidence approach**, but by no means can be used as a stand-alone tool. In cases where no sufficient complementary information is available, a conservative approach should be taken, i.e. performing a chronic toxicity test. Further analyses of existing data on acute-chronic relationships are recommended as this may enable the development of improved extrapolation tools.

Risk communication (Partner DIA)

DIALOGIK, Non-profit institute for communication and cooperation research, Stuttgart, Germany

Risk communication goes beyond public information and public relation. It needs to be seen as a necessary **complement** to **risk assessment and management**. Advertisement and packaging of messages can help to improve risk communication, but they will be insufficient to overcome the problems of stakeholders distrust in risk assessment and management institutions and to cope with the concerns, worries, or complacency of consumers. The potential remedies to these two problems lie in a better performance of all institutions dealing with or regulating risks and in structuring the risk communication mainly as a two-way communication process.

The shift from hazard assessment to risk-driven approaches has to be "communicated, communicated, communicated" in a **dialogue with the**

OSIRIS Results Highlights

stakeholders. Transparency, case studies and user-friendly, comprehensible algorithms will gain trustworthiness and credibility of integrated testing strategies (ITS).

Risk management and risk communication should be seen as parallel activities that complement each other. By carefully reviewing in-house performance, by tailoring the content of the communication to the needs of the final receivers, and by adjusting the messages to the changes in values and preferences, risk communication can convey a basic understanding for the choices and constraints of risk assessment and risk management and thus create the foundations for a trustworthy relationship between the communicator and the audience.

To ensure optimal uptake of the OSIRIS results, **end-users in industry and regulatory authorities** are **closely involved** in monitoring and in providing specific technical contributions to this project.

The OSIRIS risk communication programme needs to address the following major objectives:

- to explain the concept of probability and stochastic effects
- to clarify the potential and constraints of alternative experimental methods and techniques to generate and interpret data
- to enhance the trustworthiness and reliability of non-testing data
- to improve the credibility of the agencies, institutions and industry that provide risk information and non-testing data.

The following practical steps are recommended:

- communication of paradigm shift and scientific aspects, communication of benefits and risks (costs) of ITS
- continuous dialogue with the stakeholders
- application to case studies
- increase of the acceptance of regulatory authorities
- evaluation of the animal welfare valuation and ITS acceptance.

Binary classification models for endocrine disruptor effects (Partner IRFMN)

Istituto di Ricerche Farmacologiche, Laboratory Environmental Chemistry and Toxicology, Milan, Italy

Within REACH **endocrine disrupters** (EDs), i.e. substances interfering with the function of the endocrine system, belong to the group of chemicals of particular concern. Focusing on **estrogen receptor** (ER) mediated effects, new classification models to screen large databases for potential ED activity have been generated.

A dataset of > 800 compounds with experimentally determined receptor binding (Relative Binding Affinity to estradiol – RBA) and reporter gene assay (Relative Activity to estradiol – RA) of human ER alpha has been used to discriminate active (any determined activity) versus inactive (no detectable activity) compounds. The focus was on multiple endpoints to better characterise the potential of EDs evaluating both binding and transcriptional activity.

Robust models were derived and validated using different modelling approaches: classification trees (CART), decision forest (DF), adaptive fuzzy partition (AFP), support vector machines (SVM) and multi layer perceptron neural network (MLP). Only descriptors computed on the basis of the bidimensional structure have been included in the analysis to allow for a fast screening. Particular attention was paid to the balance between sensitivity and specificity of the models.

For all **RBA methods** accuracy was ≥ 85% on three subsets (training, validation and test set). Models obtained with CART and AFP seem to be preferable, with similar performances as others but being based on a more intuitive syntax codified in simple "if/then" rules.

For **RA methods** it was possible to reach an accuracy ≥ 80% with all methods on three subsets (training, validation and test sets). Due to the low proportion of active compounds in the dataset (25%), compared to RBA models, the RA models suffer from a greater difference between sensitivity and specificity. Overall, RA models obtained with AFP and MLP seem to perform better. As in the case of RBA models, nArOH (number of phenolic rings) and MLOGP (Moriguchi LogP) were often selected as relevant descriptors.

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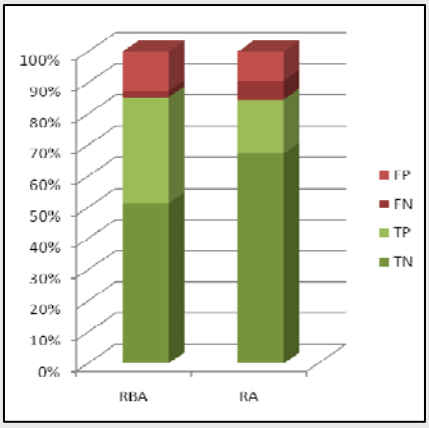
A **combination of models** has also been explored to decrease the number of false negatives (see figure).

In the context of using qualitative/quantitative structure-activity relationship (QSAR) models as a first level screening of chemicals for possible interferences with the estrogenic pathway, particular attention was paid to **avoid false negatives**, since false positives can be identified later by a subsequent tier of experimental tests.

Depending on the applicative context it is better to use the individual or combined model bearing in mind their different behaviour with respect to false negatives.



Percentage of false positive (FP), false negative (FN), true positive (TP) and true negative (TN) compounds for the combined model on the test set compounds.

Additional information: Roncaglioni A, Piclin N, Pintore M, Benfenati E 2008. Binary classification models for endocrine disrupter effects mediated through the estrogen receptor. SAR QSAR Environ. Res. 19 (7–8), 697–733

Reliability, relevance and applicability domain of alternative methods for skin and eye irritation (Partners JRC-ECVAM, LMJU)

JRC, IHCP, European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy
School of Pharmacy and Chemistry, Liverpool John Moores University, UK

A comprehensive catalogue of ***in vitro*** test methods and ***in silico*** **models** available for predicting eye and skin corrosion/irritation has been compiled. This includes an evaluation of the **reliability** and **relevance** (predictive capacity) of each of the test methods or standard operation procedure (SOP), and an evaluation of the **applicability domain** (AD) where available.

For the *in vitro* methods, the investigation was based on a comprehensive analysis of the literature and in-house information available at ECVAM. Data were compiled in a pre-defined reporting template to allow easy comparison between models.

With regard to **skin irritation**, ten *in vitro* test methods were evaluated, four methods being reconstituted human skin models, and six approaches either culturing keratinocytes or skin explants/organs. In addition, seven *in silico*/(quantitative) structure-activity relationship ((Q)SAR) models were reviewed, including a publicly available expert system, two commercially available models and four so-called local models.

Seventeen *in vitro* test methods were appraised for **eye irritation**. Among these were four organotypic methods, based on isolated animal eyes (bovine, chicken, rabbit) and the chorio-allantoic membrane of fertilised hen's eggs. In addition to these traditional tests, three reconstituted human tissue models (two currently undergoing validation at JRC-IVM) and four cell-based assays were evaluated. Furthermore, fourteen *in silico*/(Q)SAR models were evaluated.

This report allows the **identification of data gaps** which exist with regard to the parameters reliability, relevance and applicability domain analysed. For *in vitro* models, specifications concerning the applicability domain are often sparse. In addition, there is a variety of different approaches to describe the predictivity (relevance) of the assay.

The report serves as a fundamental evaluation of the current status of empirical testing methods and estimation techniques for skin and eye irritation. Based on this appraisal, a systematic assessment of the most promising methods will be generated in order to aid evidence-based construction of **intelligent testing strategies** (ITS) or modification of existing ITS for skin/eye irritation, e.g. as in the REACH guidance.

Conference Calendar: OSIRIS-related Events

Final MODELKEY conference
30 November – 2 December 2009, Leipzig, Germany
http://www.modelkey.ufz.de/conference

EMEC10 – 10th European Meeting on Environmental Chemistry
1 – 5 December 2009, Limoges, France
http://www.unilim.fr/emec10/

International Meeting on Health & Environment: Challenges for the Future
9 – 11 December 2009, Rome, Italy
http://www.iss.it/imhe-2009/

ChemCon Europe 2010
2 – 5 March 2010, Prague, Czech Republic
http://www.chemcon.net/upcoming_conferences.html

SOT 2009 – 49th Society of Toxicology Annual Meeting
7 – 11 March 2010, Salt Lake City, Utah, USA
http://www.toxicology.org/AI/MEET/AM2010/index.asp

Environmental Toxicology 2010 – Third International Conference on Environmental Toxicology
4 – 6 May 2010, Limassol, Cyprus
http://www.wessex.ac.uk/10-conferences/environmental-toxicology-2010.html

SETAC Europe 20th Annual Meeting
23 – 27 May 2010, Seville, Spain
http://seville.setac.eu

14th International Workshop on Quantitative Structure-Activity Relationships (QSARs) in Environmental Sciences
24 – 28 May 2010, Montreal, Canada
http://www.qsar2010-montreal.com/

ICCS 2010 – International Conference on Computational Science
31 May – 2 June 2010, Amsterdam, The Netherlands
http://www.iccs-meeting.org/

IUTOX-2010 – XII International Congress of Toxicology
11 – 15 July 2010, Barcelona, Spain
Spanish Association of Toxicology (AETOX)
EUROTOX in the name of the International Union of Toxicology (IUTOX)
http://gestion.pacifico-meetings.com/www/iutox2010/

3rd EuCheMS Chemistry Congress
29 August – 2 September 2010, Nürnberg, Germany
European Association for Chemical and Molecular Sciences
http://www.euchems-congress2010.org/ecc.htm

16th Congress on Alternatives to Animal Testing – Linz 2010
16th International Congress on In Vitro Toxicology - ESTIV 2010
2 – 4 September 2010, Linz, Austria
http://www.eusaat.org/index.php/2010

Risk Analysis 2010 – 7th International Conference on Computer Simulation in Risk Analysis and Hazard Mitigation
13 – 15 September 2010, Algarve, Portugal
http://www.wessex.ac.uk/10-conferences/risk-analysis-2010-3.html

18th European Symposium on Quantitative Structure-Activity Relationships
19 – 24 September 2010, Rhodes, Greece
http://www.euroqsar2010.gr/

Preview of more 2010 events :
www.osiris-reach.eu > Events and Activities > Related Events

Responsible for the OSIRIS Newsletter : Dr. Andrea Richarz
andrea.richarz@ufz.de

Helmholtz Centre for Environmental Research — UFZ, Department of Ecological Chemistry, Permoserstraße 15, 04318 Leipzig, Germany

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