Environmental ChemOinformatics

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ECO - Minutes of the 1st Winter School
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ECO - Minutes of the 1st Winter School

21 to 25 February 2011, Hochschule Fresenius, Idstein

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Attendance of project leaders and manager

1. Igor Tetko (Helmholtz Zentrum Muenchen - German Research Center for Environmental Health (GmbH), HMGU, **chair**)
2. Eva Schlosser (HMGU), project manager
3. Karl-Werner Schramm (HMGU, Germany)
4. Thomas Knepper (Hochschule Fresenius gemeinnützige Gmb, HSF, Germany)
5. Willie Peijnenburg (Leiden University, LU, The Netherlands)
6. Jan Hendriks (Radboud University Nijmegen, RU, the Netherlands)
7. José M. Navas (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, INIA, Spain)
8. Tomas Öberg (Linnaeus University, LnU, Sweden)

Participants of the 1st Winter School

9.	Ahmed Abdelaziz	Helmholtz Zentrum München
10.	Rajesh Rathore	Helmholtz Zentrum München
11.	Kamel Mansouri	University of Milano-Bicocca
12.	Faizan Sahigara	University of Milano-Bicocca
13.	Ian Dimzon	Hochschule Fresenius
14.	Isabel O'Connor	Radboud University Nijmegen
15.	Alessandra Pirovano	Radboud University Nijmegen
16.	Oleksandra Ieromina	University of Leiden
17.	Lan Song	University of Leiden
18.	Tobias Lammel	National Institute for Agricultural and Food Research and Technology
19.	Tobias Frömel	University of Leiden
20.	Marta Llorca	Hochschule Fresenius
21.	Mona Conolly	National Institute for Agricultural and Food Research and Technology

External participants

22.	Sascha Klein	Hochschule Fresenius
23.	Stefan Brandmaier	Helmholtz Zentrum München
24.	Vanessa Gellrich	Hochschule Fresenius
25.	Jutta Müller	Hochschule Fresenius
26.	Heike Weil	Hochschule Fresenius
27.	Griselda García	University of Oviedo
28.	Monika Gajewska	Silesian University of Technology

Attendance of associated participants

29.	Vladyslav Kholodovych	University of Medicine and Dentistry of New Jersey
30.	Frank Westad	CAMO Software AS
31.	Volker Mostert	Dr. Knoell Consult GmbH

Agenda of the 1st Winter School

Programme of 1st Winter School of ECO

MONDAY, 21.FEB.		
09:00 - 09:15	**Welcome by the organisator and the projectmanager**	HS A_E.01
Practical Lessons		CR A_2.01
09:15 - 12:30	Training in PLS methods *Dr. Frank Westad, CAMO Software AS, Norway* · Introduction: Science, validation, significance vs. relevance · Visualising data – the basic tools · The mother of all multivariate methods: Principal Component Analysis (PCA) - theory and examples · Outlier detection in PCA	
12:30 - 13:00	Lunch Break	Cafeteria
13:00 - 17:00	Training in PLS methods *Dr. Frank Westad, CAMO Software AS, Norway* · Hands-on exercise, PCA · Multivariate regression – theory and examples - Multiple Linear Regression (MLR) - Principal Component Regression (PCR) - Partial Least Squares Regression (PLSR) · Hands-on exercise, regression · Prediction · Summary: Steps in multivariate regression	CR A_2.01
17:00	End	
TUESDAY, 22.FEB.		
Practical Lessons		CR A_2.01
08:30 - 12:30	Training in PLS methods *Dr. Frank Westad, CAMO Software AS, Norway* · Variable selection · Multivariate regression: More hands-on 1. Choosing validation scheme 2. Decide on transformation of the original data 3. Modelling 4. Outlier detection 5. Prediction · When do we need non-linear methods? · Introduction to supervised classification methods 1. PCA as a classification method 2. PLS Discriminant Analysis (PLS-DA) 3. Support Vector Machines · Summary, questions and answers (30 mins)	
12:30 - 13:00	Lunch Break	Cafeteria
Theoretical Lessons		HS A_E.01
13:00 - 15:00	Introduction to sampling on Wednesday and analytical exercises on Thursday *Prof. Thomas Knepper, Hochschule Fresenius, Idstein*	
Practical Lessons		CR A_2.01
15:00 - 18:00	Advanced training in OCHEM *Dr. Igor Tetko, Helmholtz-Zentrum München*	
18:00	End	
18:30 - 23:00	Fellows meeting at "Brauhaus Idstein" *only for fellows*	

WEDNESDAY, 23.FEB.		
Excursion		
09:15 - 10:00	Bus ride to Schierstein	Meeting Point ZOB
10:00 - 14:00	Visit of Wasserwerk Schierstein including sampling	
11:00 - 13:00	Systematic monitoring of pesticide transport and fate in water and soil systems, *Prof. Thomas Knepper, Hochschule Fresenius, Idstein*	
15:00	Arrival in Nieder-Walluf	
15:00 - 16:00	Joint lunch at "Bug's Weinschänke" in Nieder-Walluf	
16:00	Departure Nieder-Walluf	Meeting Point KD
16:45	Arrival at Idstein	
THURSDAY, 24.FEB.		
Practical Lessons		Laboratories
09:00 - 12:30	Determination of pharmaceuticals with Liquid chromatography tandem mass spectrometry (LC-MS/MS) after solid phase extraction Extraction of selected pharmaceuticals by solid phase extraction (SPE), Prof. Thomas Knepper and team, Hochschule Fresenius, Idstein Selected pharmaceuticals: - Antibiotics sulfamethoxazole and trimethoprim, - Betablockers metoprolol and propranolol, - Antiepileptic drug carbamazepine, - Painkiller phenazon, SPE: - Filtration of surface water samples - Adding internal standard - Conditioning the SPE cartridges - Extraction - Drying the SPE cartridges - Elution - Evaporation of the extracts	
12:30 - 13:00	Lunch Break	Cafeteria
Practical Lessons		Laboratories
13:00 - 17:45	Analysis with LC-MS/MS, *Prof. Thomas Knepper, Hochschule Fresenius, Idstein* Instrument: 3200 Q Trap® LC/MS/MS (Applied Biosystems, Foster City, USA) LC-MS/MS method development: - Infusion of one selected pharmaceutical via syringe pump - Optimisation of instrumental parameters (declustering potential, entrance potential, collision energy, collision cell entrance/exit potential) by continuous flow injection. - Discussion of fragmentation routes during collisional-induced dissociation (CID) - Development of a multiple reaction monitoring (MRM) method for all selected pharmaceuticals - Measuring the samples in MRM mode overnight.	
17:45 - 18:00	Evaluation of Passive Sampling with SBSE (Autumn School) *Prof. Karl-Werner Schramm, Helmholtz-Zentrum München*	
18:00 - 20:00	General Assembly *only for Project leaders and -manager*	BR A_E.02

FRIDAY, 25.FEB.		
Practical Lessons		**Laboratories**
09:00 - 12:30	Analysis with LC-MS/MS, *Prof. Thomas Knepper, Hochschule Fresenius, Idstein* - Calibration - Quantification - Validation	
12:30 - 13:30	Lunch Break	Cafeteria
Theoretical Lessons		**HS A_E.01**
13:30 - 15:00	Scientific discussion round of fellows *Dr. Eva Schlosser, Helmholtz-Zentrum München*	
15:00 - 16:00	Scientific project management *Dr. Eva Schlosser, Helmholtz-Zentrum München*	
16:00 - 17:00	Come together / Farewell	
17:00	**Departure**	

Opening of the 1st Winter School

Prof. Knepper opened the 1st Winter School, and presented the program together with Dr. Schlosser. With good wishes for a successful school, the meeting started.

Training in multivariate data analysis, focusing on PLS methods (1½ days)

During the training sessions by ECO associate partner CAMO Software AS, represented by Dr. Westad, the participants were introduced into multivariate data analysis, starting with principal component analysis (PCA) and continuing with multivariate calibration (MLR, PCR and PLSR). The theory was covered in lectures followed by hands-on exercises for PCA and multivariate regression. This training also covered related subjects such as variable selection, non-linear and classification methods.

Advanced training in the Online Chemical Modelling Environment (OCHEM)

HMGU provided training in advanced use of OCHEM for QSAR/QSPR work. The lectures covered key steps such as: Compounds selection, descriptors selection, building the QSAR model, and methods for validating the model. This workflow was subsequently demonstrated in OCHEM. In addition, approaches to experimental design with partial least squares approaches were presented with implementation in OCHEM.

Excursion to Wasserwerk Schierstein and sampling exercise (1 day)

A visit was made to the waterworks at Schierstein including sampling. Here, a separate lecture was also devoted to the systematic monitoring of pesticide transport and fate. The samples collected were subsequently examined and evaluated in the analytical sessions. Besides explaining the regulatory affairs, emphasis was made to explain sampling strategies and obstacles occurring during analysis of micro pollutants. On top, focus was given upon polar and recalcitrant compounds including transformation products.

Analytical sessions with focus on LC-MS/MS (1½ days)

Hochschule Fresenius, Prof. Knepper, provided several training sessions and analytical exercises with liquid chromatography tandem mass spectrometry. These sessions covered sample preparation using solid-phase extraction methods, LC-MS/MS method development including optimisation of instrumental parameters, calibration, quantification and validation.

Passive sampling with SBSE was covered in a lecture by HGMU, Prof. Schramm.

Scientific discussion round and project management (½ day)

During the last day a scientific discussion round was carried out to enhance the cooperation within the network and to advance the fellows' ability in autonomous scientific working. Computational and experimental groups outlined the possibilities for common projects. One important point was the discussion where each fellow should pass her / his secondment as foreseen in the grant agreement. Especially secondments with associated participants were reconsidered.
Finally a lecture was given on scientific project management by Dr. Schlosser to prepare the fellows for the challenges ahead in carrying out their respective projects.

General Assembly

An evening was devoted to a general assembly. Guest was Dr. Mostert from the company Dr. Knoell Consult GmbH. Together with him further details of the next Summer School were discussed, particularly the introduction of the fellows into the elements of REACH. Another important point of the discussion was the occupation of the remaining STR-months. It was decided unanimously, to offer Mona Conolly, fellow at INIA, further 10 months in Madrid with Dr. Navas and beyond months either in Munich, in the group of Prof. Schramm, or in Leiden, in the group of Prof. Peijnenburg. Combined fellowships are also considered for the candidates Tine Ringsted and Eva Giagloglou. At last the secondments of the fellows were planned, both internally so even with associated partners. It has been decided to ask about the

fellows' requirements and to prepare an accurate schedule for all secondments until the mid-term review meeting.

Social events and networking

During the fellows meeting as well as on the excursion and laboratory exercises the fellows had multiple opportunities to get to know each other. This part of the schools continue be of great importance as the success of the ECO ITN programme will depend on a good working network.

Evaluation of the 1st Winter School

On the last day of the school an evaluation of the school was implemented by the local organizer and the project manager. Each part of the programme was evaluated separately. The degree of satisfaction was 90-100%. The two main parts of the school, training in multivariate data analysis and chemical analytical methods, received 100% satisfaction. All lecturers were highly appreciated.

Conclusions and outlook

In summary, the school was a great success and each fellow is looking forward to the 2nd Summer/Autumn School in September and to meet each other again.

HelmholtzZentrum münchen
German Research Center for Environmental Health

Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Project report 1 /2011

29 March 2011

Environmental toxicity: Insilico prediction accounting for bioavailability and mechanism of action

Early stage researcher:
Ahmed M. Abdelaziz

Project supervisor:
Igor V. Tetko

Research Institution:
HelmholtzZentrum Muenchen

Project description: The accuracy of prediction of in vivo toxicity endpoints of chemicals is expected to dramatically increase by an incorporation of information about mechanisms of action of molecules. Indeed, it is generally recognized that the toxicity of chemicals is the result of their influence on several toxicity mechanisms, which include for example the Wnt, Delta-Notch, Ras, TGF-b, and Hedgehog pathways. Therefore, the accurate use of the information about mechanism of action of molecules on these pathways (using, e.g. in vitro measurements or predicted with corresponding in silico models), and grouping of molecules according to their mode of action can increase the accuracy of in vivo toxicity predictions as well as provide a clear mechanistic explanation of the toxicity of chemicals. The information about the bioavailability of molecules can be also important to have better interpretation of the in vitro and in vivo correlations. This PhD will focus on the use of mechanism of action of molecules and will be complemented by work on bioavalability of molecules that is a topic of the second PhD work in our center within the Marie Curie Initial Training Network "Environmental ChemOinformatics". The in vitro data from ToxCastTM project will be used. On the latest phase, the project will develop WWW tools for prediction of in vivo toxicity using the in vitro measurements and structural information of molecules.

Break-down:

Concept: The bioavailability governs *in vivo* toxicity. The compounds that are not bioavailable cannot act on their biological targets even if they act on the *in vitro* assays. The pharma industry uses BCS classification to categorize chemical compounds according their solubility and permeability. I will develop models to categorize chemical compounds according BCS concept, which may allow observing better correlation between their in vitro and in vivo activities.

WP1. The oral bioavailability of compounds is a function of absorption in gastrointestinal tract at different pH. The ChemAxon descriptors allow characterizing chemical structures at different pH and could be useful to predict oral bioavailability of chemicals. Therefore, these descriptors will be implemented as part of QSAR modeling platform (On-line CHEmical Modeling environment http://ochem.eu).

Results:

Most of Chemaxon descriptors (also known as: Chemaxon calculators) were implemented in OCHEM platform and can be calculated for any set of molecules. Descriptors implemented are those that return numerical or Boolean results. Unimplemented descriptors are those which return results not suitable for modeling purposes such as molecules, formula, or fingerprints. Also calculators that require specific input parameters are not implemented. Examples of these calculators are those that check whether certain atom is asymmetric, whether 2 atom are connected, or calculate the angle between 3 specified atoms.

The implemented descriptors are divided into 7 groups: Elemental Analysis, Charge, Geometry, Partitioning, Protonation, Isomers, and Others

Descriptor Name	Descriptor class	Definition	Considers pH during calculation
atomcount	Elemental Analysis	Number of atoms in the molecule	no
exactmass	Elemental Analysis	Exact molecule mass calculation based on the most frequent natural isotopes of the elements	no
mass	Elemental Analysis	Molecule mass calculation	no
			no
averagemolecularpolarizability	Charge	Average molecular polarizability calculation	yes
formalcharge	Charge	Formal charge calculation	yes
molecularpolarizability	Charge	Molecular polarizability calculation	yes
			no
aliphaticatomcount	Geometry	Aliphatic atom count	no
aliphaticbondcount	Geometry	Aliphatic bond count	no
aliphaticringcount	Geometry	Aliphatic ring count	no
aliphaticringcountofsize	Geometry	Aliphatic ring count of size	no
aromaticatomcount	Geometry	Aromatic atom count	no
aromaticbondcount	Geometry	Aromatic bond count	no
aromaticringcount	Geometry	Aromatic ring count	no
aromaticringcountofsize	Geometry	Aromatic ring count of size	no
asymmetricatomcount	Geometry	The number of asymmetric atoms	no
balabanindex	Geometry	The Balaban index	no
bondcount	Geometry	Bond count	no

carboaliphaticringcount	Geometry	Carboaliphatic ring count	no
carboaromaticringcount	Geometry	Carboaromatic ring count	no
carboringcount	Geometry	Carbo ring count	no
chainatomcount	Geometry	Chain atom count	no
chainbondcount	Geometry	Chain bond count	no
chiralcentercount	Geometry	The number of tetrahedral stereogenic center atoms	no
dreidingenergy	Geometry	Calculates the dreiding energy of a conformer of the molecule in kcal/mol	no
fragmentcount	Geometry	Fragment count	no
fusedaliphaticringcount	Geometry	The number of fused aliphatic rings (SSSR smallest set of smallest aliphatic rings)	no
fusedaromaticringcount	Geometry	The number of fused aromatic rings (SSSR smallest set of smallest aromatic rings)	no
fusedringcount	Geometry	The number of fused rings (SSSR smallest set of smallest rings)	no
hararyindex	Geometry	Harary index	no
heteroaliphaticringcount	Geometry	Heteroaliphatic ring count	no
heteroaromaticringcount	Geometry	Heteroaromatic ring count	no
heteroringcount	Geometry	Hetero ring count	no
hyperwienerindex	Geometry	Hyper Wiener index	no
largestringsize	Geometry	Largest ring size	no
largestringsystemsize	Geometry	Largest ring system size	no
maximalprojectionarea	Geometry	Calculates the maximal projection area	no
maximalprojectionradius	Geometry	Calculates the maximal projection radius	no

maximalprojectionsize	Geometry		no
minimalprojectionarea	Geometry	Calculates the minimal projection area	no
minimalprojectionradius	Geometry	Calculates the minimal projection radius	no
minimalprojectionsize	Geometry		no
molecularsurfacearea	Geometry	Molecular Surface Area calculation (3D)	yes
plattindex	Geometry	The Platt index	no
randicindex	Geometry	The Randic index	no
polarsurfacearea	Geometry	Topological Polar Surface Area calculation (2D)	yes
ringatomcount	Geometry	Ring atom count	no
ringbondcount	Geometry	Ring bond count	no
ringcount	Geometry	Ring count	no
ringcountofsize	Geometry	Ring count of size	no
ringsystemcount	Geometry	The number of ring systems	no
ringsystemcountofsize	Geometry	Ring system count of size	no
rotatablebondcount	Geometry	Rotatable bond count	no
smallestringsize	Geometry	Size of smallest ring	no
smallestringsystemsize	Geometry	Smallest ring system size	no
stereodoublebondcount	Geometry	The number of stereo double bonds	no
szegedindex	Geometry	Szeged index	no
volume	Geometry	Calculate the van der Waals volume of the molecule	no
wienerindex	Geometry	Wiener index	no
wienerpolarity	Geometry	Wiener polarity	no
vdwsa	Geometry	Van der Waals Surface Area	yes

		calculation	
			no
logp	Partitioning	logP calculation: logP of uncharged species, or, in the case of zwitterions, logD at pI	no
logd	Partitioning	logD calculation	yes
			no
isoelectricpoint	protonationDescriptors	Isoelectric point calculation	no
			no
doublebondstereoisomercount	Isomers	The number of double-bond stereoisomers of the molecule	no
stereoisomercount	Isomers	The number of stereoisomers of the molecule	no
tautomercount	Isomers	The number of tautomers	no
tetrahedralstereoisomercount	Isomers	The number of tetrahedral stereoisomers of the molecule	no
			no
acceptorcount	Others	Hydrogen bond acceptor atom count in molecule	yes
acceptorsitecount	Others	Hydrogen bond acceptor multiplicity in molecule	no
donorcount	Others	Hydrogen bond donor atom count in molecule	yes
donorsitecount	Others	Hydrogen bond donor multiplicity in molecule	yes
hmopienergy	Others	HMO Pi energy	yes
pienergy *	Others	Pi energy	yes
msacc	Others	Hydrogen bond acceptor average multiplicity over microspecies by pH	no
msdon	Others	Hydrogen bond donor average multiplicity over microspecies	no

		by pH	
resonantcount	Others	The number of resonant structures	no

pH descriptors

Calculation of some descriptors requires consideration of pH value. User is allowed 3 options for pH:

- All: This calculates the value of the descriptor over the pH range from 0 to 14 taking 1 pH unit increments at a time. Additionally, the descriptor value at pH 7.4 is calculated.
- Specific value: calculates the value of the descriptor at the speicifed pH.
- Specific range: calculates the value of the descriptor over the pH range specified between "from" until "to" taking pH unit increments equal to the value specifid in "step". Additionally, the descriptor value at pH 7.4 is calculated.

Descriptors, which consider pH value during their calculation, are: averagemolecularpolarizability, formalcharge, molecularpolarizability, molecularsurfacearea, polarsurfacearea, vdwsa, logd, acceptorcount, acceptorsitecount, donorcount, donorsitecount, hmopienergy, pienergy

WP2: QSAR models for prediction of solubility and oral bioavailability of chemical compounds will be developed using data available at the OCHEM platform.

Results:

Models were developed based on literature data published in the following articles:

Articles	Compounds
MDCK (Madin-Darby canine kidney) cells: A tool for membrane permeability screening. [1]	55
Molecular hashkeys: a novel method for molecular characterization and its application for predicting important pharmaceutical properties of molecules. [2]	20
In silico ADME modeling 3: Computational models to predict human intestinal absorption using sphere exclusion and kNN QSAR methods [3]	174
CODES/neural network model: A useful tool for in silico prediction of oral absorption and blood-brain barrier permeability of structurally diverse drugs [4]	28
Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. [5]	25
ADME evaluation. 2. A computer model for the prediction of intestinal absorption in humans. [6]	49

Toward minimalistic modeling of oral drug absorption.	85
Experimental and computational screening models for the prediction of intestinal drug absorption.[7]	20
Functional role of P-glycoprotein in limiting intestinal absorption of drugs: contribution of passive permeability to P-glycoprotein mediated efflux transport.[8]	88
Prediction of human intestinal absorption of drug compounds from molecular structure. [9]	86
Rate-limited steps of human oral absorption and QSAR studies. [10]	237
Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption. [11]	21

Models were developed using Multiple linear regression (MLRA) and Artificial neural networks (ANN). The chemaxon descriptors, which were integrated in **WP1**, were used together with other descriptor packages like AlogPS, and CDK descriptors.

Model developed between Human Intestinal absorption and the aforementioned in silico descriptors had R^2 value of 0.62 and $q^2 = 0.61$. In order to improve the model and investigate the weaker points in bioavailability predictions based on mechanistic approach the following models are being developed to act as parts in a holistic bioavailability approach:

- QSAR model for plasma protein binding
- QSAR model for plasma to blood partition
- QSAR model for CACO-2 permeability
- QSAR model for hepatic clearance (by hepatic suspension cells)

WP3: Integration of data available from TOXCAST project to be used within the project. The data will be uploaded from PubChem/ACToR database.

Results: A complete copy of ACTOR system was downloaded and installed locally.

ACToR is EPA's online warehouse of all publicly available chemical toxicity data and can be used to find all publicly available data about potential chemical risks to human health and the environment. ACToR aggregates data from over 500 public sources on over 500,000 environmental chemicals searchable by chemical name, other identifiers and by chemical structure.
The data warehouse:

- Allows users to search and query data from other EPA chemical toxicity databases including:
    - ToxRefDB (30 years and $2 billion worth of animal toxicity studies).
    - ToxCastDB (data from screening 1,000 chemicals in over 500 high-throughput assays).
    - DSSTox (provides high quality chemical structures and annotations).

A framework is being built using Knime over the whole database to be able to build models correlating in vivo data from ToxRefDB with in vitro data from ToxCastDB. This framework will be the base for testing the effect of bioavailability and biochemical pathways on quality of correlation.

Milestone 1: QSAR models were developed for classification of chemicals. Analysis and categorization of molecules from ToxCast project according to their solubility and permeability.

Refrences

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Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Project report 1 /2011

30 March 2011

Polymer and Polymer Degradation Products in Aqueous Environment

Early stage researcher:
Ian Ken D. Dimzon

Project supervisor:
Thomas P. Knepper

Research Institution:
Institute for Analytical Research
Hochschule Fresenius

Quarterly Report 1/2011
January – March 2011

Polymers and Polymer Degradation Products in Different Aqueous Environment

Summary:

This quarter was mostly used to optimize the mass spectrometric methods for the characterization of chitosan and organosilicone oligomers. The researcher can now detect low molecular weight (lower than 2000 Mn) polymers using matrix-assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) mass spectrometric (MS) techniques with higher reproducibility. The biodegradation study on chitosan was not started because the methods for higher molecular weight polymers were not yet developed.

During this quarter, the researcher's home institute, the Institute for Analytical Research - Hochschule Fresenius (IFAR HF) hosted the first Winter School of ECO. The lectures and practical trainings deepened the fellows' understanding of basic chemometrics, liquid chromatography and mass spectrometry in the context of environmental analysis.

I. Background (August – December 2010)

The previous quarter (August – December 2010) was spent for the researcher's orientation and initial trainings. Preliminary experiments, mostly on the MALDI MS of chitosan oligosaccharide were done to set the direction of the researcher's thesis. The first Autumn School provided the researcher a venue to understand the whole ECO Project and a chance to get to know the other fellows in the program.

II. Continuation of the MALDI MS Experiments

MALDI MS is being developed as a tool to detect polymers and their degradation products. Since the previous quarter, the researcher has been optimizing the different parameters to increase the sensitivity of the instrument to heavy molecules: sample spot preparation, cationization, fractionation and matrix to polymer ratio.

The researcher was able to develop a reproducible method for the chitosan oligosaccharide (Mn < 2000 Da) with 2,5-dihydroxybenzoic acid as matrix compound. The method was also applicable to other polar oligomers like the organosilicone oligomers and the polyethylene glycol.

While the method worked well with the chitosan oligosaccharide, detection of large molecular weight chitosan (Mn > 2000 Da) is still being worked on. The low sensitivity of the MALDI-MS technique at the high mass to charge (m/z) region can be due to two factors: vaporization of the polymer and the detector response. It would be necessary to detect high molecular weight chitosan before proceeding with the degradation experiments.

III. Preliminary runs on the ESI-MS

The Applied Biosystems AP2000 ESI-Triple Quad MS is the institute's newest instrument. It was installed last December 2010 and started being operational last January 2011. The ESI method to detect chitosan and organosilicone oligomers is being developed to compliment the MALDI MS technique. With the ESI technique, it will be possible to quantify the polymers and their degradation products.

The researcher had an initial technical training on the ESI MS. The organosilicone oligomer was used as sample polymer to explore the different measurement modes of the instrument. After the training, the researcher started to

optimize the ESI scan and multiple ion modes to study the distribution of organosilicone oligomers. The developed ESI MS method was then tested for applicability to chitosan oligosaccharide. Unlike in the MALDI, ESI mass spectrum generated not only singly charged ions but multiply charged ions as well (See Appendix A).

IV. Work plan and Gantt chart

The planned activities for the quarter as specified in the researcher's Gantt chart were mostly met. Both MALDI-MS and ESI-MS were optimized to characterize chitosan oligomers and organosilicone oligomers. There is however a problem in the detection of higher molecular weight polymers. The researcher is yet to identify the cause of the problem and modify the parameters accordingly.

The work package on the degradation of chitosan was not started yet because of the problem stated above. In the degradation study, higher molecular weight chitosan (Mn > 2000 Da) will be exposed to different physico-chemical and microbiological conditions resulting to its degradation. MALDI and ESI will be used to monitor the changes in the mass distribution of chitosan as it degrades.

V. 1st Winter School

The researcher's home institute, the IFAR HF, hosted the first Winter School of ECO last 21-25 February 2011 in Idstein, Germany. During the first two days, the invited speaker from CAMO software, Frank Westad, gave an extensive lecture on basic chemometrics and a short training in using the Unscrambler® Software. The speaker talked about Principal Component Analysis (PCA), Principal Component Regression (PCR) and Partial Least Squares (PLS). In the afternoon of Tuesday, Prof. Thomas Knepper gave an introductory lecture on liquid chromatography and mass spectrometry and briefed the fellows on their tasks for the next three days.

On the third day of the school, the fellows went on a field trip to Hessen Wasser, a water treatment facility in Wiesbaden, Germany. During the trip, Prof. Knepper gave a talk on environmental monitoring and its importance. After the talk, the fellows had a walk to the Rhein River to take water samples for the practical training the next day.

The last two days of the winter school was devoted to the practical training using the ESI MS-MS to determine the pharmaceutical compounds in water sample. The researcher, as part of the IFAR team, helped in the organization of the practical training. He supervised the preparation and enrichment of the water samples by solid phase extraction. He learned how to conduct training and supervise practical courses. The other fellows, meanwhile, learned about analytical chemistry techniques.

Overall, the winter school was a success. It had been another opportunity for the fellows to come together, build networks and learn new things.

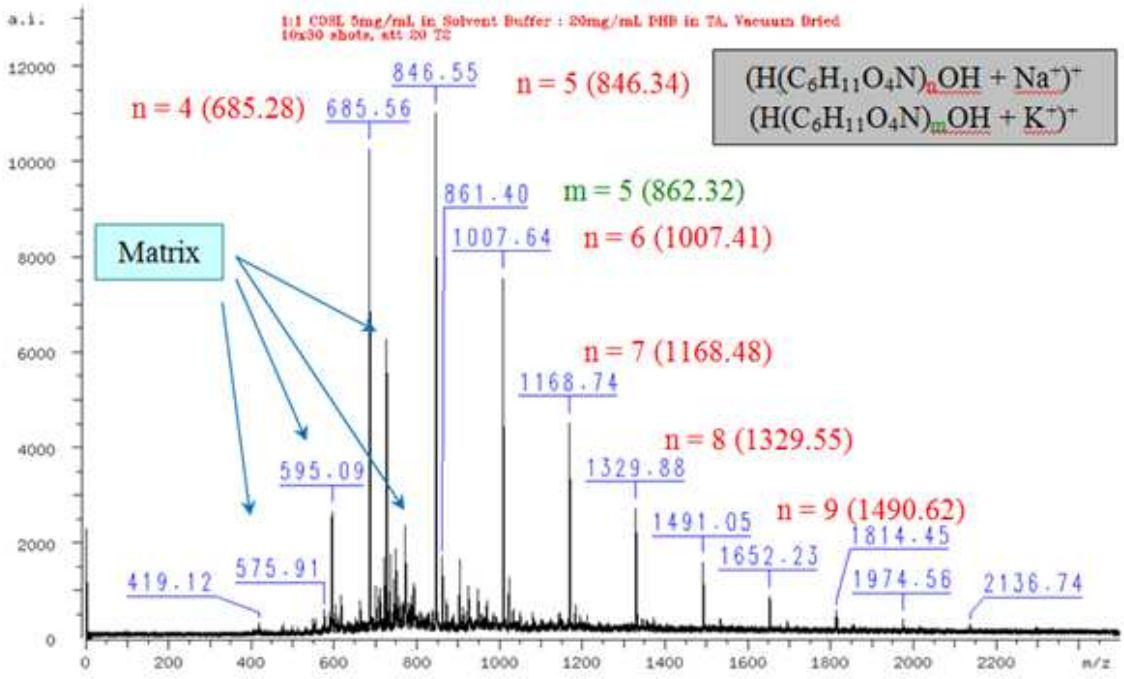
VI. For the next quarter

The experiments on optimizing the MALDI and ESI conditions for the detection of high molecular weight polar polymers will be continued. Degradation tests on chitosan will be done next.

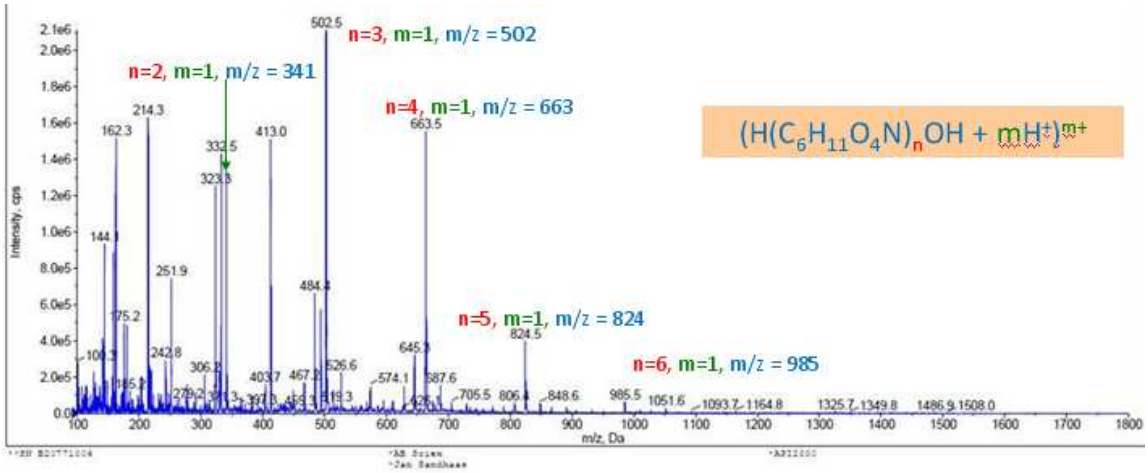
Meanwhile, the researcher submitted abstracts of his work to two scientific conferences in the Philippines and in Italy. The abstracts (see Appendices B and C) were accepted for poster presentation.

Appendix A
MALDI and ESI Mass Spectra of Chitosan Oligosaccharide

MALDI Mass Spectrum



ESI Mass Spectrum



Appendix B
Abstract for the 26th Philippine Chemistry Congress in Cebu City, Philippines on 13 – 15 April 2011

Characterization of Chitosan Oligosaccharide by GPC, MALDI-TOF MS and ESI-MS-MS

Ian Ken D. Dimzon and Thomas P. Knepper
Institute for Analytical Research
Hochschule Fresenius – University of Applied Sciences
Limburger Str 2, 65510 Idstein, Germany

Abstract

Chitosan oligosaccharides have been widely used in the pharmaceuticals and cosmetics industries because of their high solubility and their bioactivity. These properties are dependent on the oligosaccharides' molecular weight distribution and degree of deacetylation.

In this study, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and electrospray ionization tandem mass spectrometry (ESI-MS-MS) were used to determine the exact molecular weight distribution and to obtain information regarding the chemical structure and composition of a chitosan oligosaccharide sample.

The derived molecular weight distributions from the mass spectrometry (MS) methods were compared to those derived from gel permeation chromatograpy (GPC) methods. The MS-derived distributions were dependent on method optimization parameters while the GPC-derived distributions were dependent on the type of molecular weight standards used.

The combination of MALDI-TOF MS and ESI MS-MS provided additional information on the composition and degree of deacetylation of the molecules. The combined mass spectrometric techniques would be vital in elucidating the structure-reactivity relationships of chitosan oligosaccharides.

Keywords: Chitosan oligosaccharide, MALDI, ESI, GPC

Appendix B
Abstract for the SETAC Europe 21st Annual Meeting in Milan, Italy on 15 – 19 May 2011

Detection of Chitosan Oligosaccharide by MALDI-TOF MS

Ian Ken Dimzon, Marco Bernhard and Thomas Knepper
Institute for Analytical Research
Hochschule Fresenius, University of Applied Sciences
Limburger Str. 2, 65510 Idstein, Germany

Abstract

Because of their increasing use in the pharmaceuticals and cosmetics industry, there is a need to study the fate of soluble, bioactive chitosan oligosaccharides and their degradation products in water. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) offers a fast but highly selective way of detecting oligomers like chitosan.

In this study, the parameters in the MALDI-TOF MS analysis of chitosan oligosaccharide lactate were optimized to improve sensitivity of the method. 2,5-Dihydroxybenzoic acid (DHB) was used as matrix. The spots prepared by vacuum drying gave more intense and more reproducible analyte ion signals. Compared to the spots prepared by drop and dry and by quick and dirty techniques, vacuum dried spots were thinner, less crystalline, more evenly spread and with fewer hot spots.

Similar to that of other polymers, the presence of cations is necessary in the formation of ionized chitosan, mostly as sodium adducts. For better signal, however, the amount of salt was optimized by partial desaltation using Amberlite mixed-bed ion exchange resin. Fractionation by size exclusion chromatography improved the signal of the higher molecular weight chitosan.

Topic: B05-Environmental fate and exposure of Pharmaceuticals and Personal Care Products (PPCPs)

Keywords: MALDI, Chitosan

Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Project report 1 /2011

30 March 2011

Impact of chemical stressors on aquatic ecosystems under field conditions

Early stage researcher:
Ieromina Oleksandra

Project supervisors:
Prof. dr. ir. W.J.G.M. Peijnenburg
Dr. ing. M.G. Vijver
Prof. dr. G.R. (Geert) de Snoo

Research Institution:
Institute of Environmental Sciences (CML)
Leiden University

Project goals

Chemical contaminants are common in nature. Understanding and predicting impacts of contaminants on natural ecosystems is challenging for ecologists. The ecological risk assessment (ERA) of chemicals combines fundamental ecology and toxicology. Lower tiers of ERA are performed based on individual organism tests and toxicological effects on individual level. However most often protection goals of ERA concern higher organization levels – populations, communities and ecosystems. Therefore the field of risk assessment of chemicals encounters the need for high organizational level approaches.

In aquatic environment organisms are exposed to a large number of chemicals originating from different sources and further question is combined toxic effect of chemicals that co-occur or appear in mixtures. In a multiple chemical exposure, the single chemicals may act independently as in a single exposure, or a number of the chemicals may interact to form the effects of the total multiple exposure. The study of complex chemical mixtures is largely complicated by the various types of interactions existing between chemicals, and between chemicals and biological systems. In order to understand the toxicological effects of chemical mixtures, detailed information on the composition of the mixture, the mechanism of action, toxicity of each compound, as well as exposure data is required.
Potential effects of mixtures of chemicals on biota are of great concern.

The generic aim of PhD project is to assess effects of mixtures of chemicals on ecosystems in the field, whilst explicitly considering the fact that organisms are subjected to multiple stressors varying over time and over space.
The objectives of the project are:

- Estimate the effect of chemical mixtures on freshwater macroinvertebrate communities in the field (taxonomic composition and ecological trait modalities)
- Assess impact of pesticide mixture toxicity on several invertebrate species in a series of in situ experiments

Time planning

PhD project planned for September 2010 – August 2014
Literature research and formulation of research objectives: September – December 2010
Field work: March-July 2011, 2012, 2013
Training courses organized by ECO ITN and conferences are planned during the project
Internship in Hochschule Fresenius - Institute for Analytical Research scheduled for November – December 2011

Collaboration

Project planning includes collaboration with RIVM (National Institute of Public Health), Bilthoven, Netherlands; Water Management Board (Rijnland), Leiden, Netherlands

Description of Work Packages

Work Package 1

Title: Literature overview on the impact of chemicals on freshwater aquatic ecosystems (Completed)

Purpose:

- Literature research on effects of multiple stressors on aquatic ecosystems (on organism, species, population, ecosystem level)
- Overview of the main principles and methods in risk assessment of chemicals (field monitoring, mesocosms, bioassays, laboratory toxicity tests)
- Taxonomic and species trait approaches in risk assessment of chemicals in freshwater aquatic ecosystems. Biological indicators of pesticide contamination
- Pesticide usage in the Netherlands. Ecological effects of application of mixtures of pesticides. Effect of pesticides on non-target organisms
- Formulation of research objectives

Timing: September 2010 – January 2011

Work Package 2

Title: Assessment of macroinvertebrate diversity across the gradient of land use in the province Southern Holland (ongoing)

Purpose:

- Analyze historical databases on macrofauna/pesticides/water chemistry (by means of multivariate statistics, principal component analysis)
- Compare macrofauna taxonomic and trait composition in areas with different land use activities and subsequent emission of different mixtures of chemicals (flower bulb growing region, grassland, peat, forest) and soil type (sand, clay, peat)
- Estimate potential of species traits in quantifying effects of landuse on invertebrate communities in the streams. Study functional attributes of invertebrates as well as taxonomic composition along the gradient of agricultural development (nutrients, sedimentation, pesticides)

Results expected:

There are differences in macrofauna community types depending on land use activities. In grassland macrofauna diversity is enhanced and high levels of species richness are maintained. Flower bulb growing coupled with application of pesticides decreases macrofauna diversity

Timing: March 2011 – December 2011

Collaboration: Water Management Board (Rijnland)

Work Package 3

Title: Toxic stress and species traits: effects of pesticides on taxonomic and trait composition of freshwater macrofauna (ongoing)

Taxonomical and functional components of biodiversity respond differently to anthropogenic disturbance. Assessment and study of aquatic communities can be enhanced if functional characteristics are considered for taxonomic groups. For instance, intensification of land use in agricultural fields can result in diffusion of fine sediments and

organic particles into the rivers and channels. Fine material is very likely to promote deposit feeders or species that live in mud substrate so that higher number of deposit-feeders is expected in locations with extensive land use. On the other hand, feeding guild information can reflect nutrient cycling, resource processing (shredder or grazer) and trophic position (for instance, predator or omnivore). Functional attributes provide more complete understanding of ecosystem response to stress rather than solely taxonomic description.

Purpose:

- investigate if inclusion of functional characteristics of species (species traits) can improve risk assessment of mixtures of toxicants in freshwater ecosystems. To reach this aim we are going to study the impact of toxicants (pesticides) on macrofauna communities under field conditions using differential approaches (taxonomic and species traits)

In order to test this, it is needed:

- effect of pesticides on species traits of aquatic invertebrates
- linkage between traits and parameters reflecting ecosystem functioning
- recovery of invertebrate community after toxic stress and traits responsible for fast recovery

Objectives of the field work in the bulb area (Rijnland):

1. Collect macroinvertebrate samples from selected sampling locations: flower bulb fields, pastures, control sites in clean dune area (similar sediment type)
   - Taxonomic identification of the organisms in the sample
   - Assessment of selected trait categories (body size, feeding mode, respiration mode, life cycle, mobility and dispersal, number of reproduction cycles per year, form of aquatic life stage, diapause/resistance form, type of lipids, etc)
2. Measure water chemistry parameters in selected sampling locations (temperature, pH, conductivity, alkalinity, O2, nitrite, nitrate, phosphorus)
3. Measure concentration of pesticides in selected locations (OMEGAM Laboratory)

Methods:

Macrofauna collected with 500µ mesh D-frame net. Water chemistry measured in the field. Nutrients and pesticides measured in OMEGAM Laboratory.

Research area: Flower growing region in Southern Holland (on the north of Nordwijk). Field sites selected in 3 areas: flower bulb fields (fertilizers and pesticides applied), grassland (nutrients) and sandy dunes (clean water)

A large area in the Netherlands is used for flower cultivation. The sector of growing flower bulbs is among the most polluted in agricultural industry in the Netherlands. Due to intensive flower growing resulted in high yield large amounts of pesticides are applied. To preserve the plants and bulbs in a good condition, the soil and harvested bulbs have to be treated with pesticides. The spraying of pesticides occurs frequently in the period from March till May. Main pesticides applied in the area are fungicides (Carbendazim, Imazalil) and insecticides (Imidacloprid). However intensive pesticide usage is coupled with a number of side effects: direct spray in ditches, absorption to soil particles, degradation in soil and surface water, adverse effects on non-target organisms. Flowers in Southern Holland are grown mainly on sandy soil what makes the run off of pesticides to the surface water and groundwater higher than on other soil types. Coarse-textured sandy soils have

the largest pores and the fastest permeability. Sandy soils have less organic matter and very low sorption potential. Therefore the chemicals are nor retained and degraded in the soil but are washed into ground water and ditches, leading to water contamination and harmful effects on aquatic organisms.

A lot of research has been done on the effects of individual pesticides on biota, but less is known about the effects of pesticide mixtures. In nature organisms are exposed to combinations of contaminants and pesticides occur usually as complex mixtures. Observed effects of pesticides from the same class are often additive in nature. When there is combined exposure to pesticides within the same mechanism group, the principle of dose addition for compounds exhibiting simple similar action is applied. For instance, mixtures of herbicides characterized by different modes of action show concentration additivity in their toxicity effects. Research described is focusing of effects of mixture toxicity of pesticides on aquatic invertebrates that represent important component of aquatic ecosystems and are very sensitive for pesticide contamination.

Results expected:
Trait composition of aquatic invertebrates as well as linkage with ecosystem function will be linked to pesticide application. We expect that traits referring to population resilience (body size, generation time, reproduction mode) will show significant correlation with increasing contamination level. We expect that abundance of invertebrates will decrease with higher loading of contaminant.
The linkage towards nutrient concentrations will be the other way around: higher nutrient concentration results in higher food availability and increased abundance. However high nutrient levels can cause extensive algal growth that results in hypoxia and reduction of invertebrates abundance.
We expect that allocation of biomass among different size classes of macroinvertebrate community will change in response to pesticide contamination and nutrient loading.
Time planning
Field work will be executed in March-June 2011, March-June 2012 and March-June 2013
Costs
Collaboration: Water Board (Rijnland), OMEGAM Laboratory

Work Package 4

Title: Verification of species trait theory in the field. Effect of mixtures of pesticides on reproduction and growth rate/body size of 4 Amphipoda species

Purpose:
- Investigate effect of pesticides on reproduction and body size of aquatic invertebrates in a series of field enclosure experiments with 4 Amphipoda species ranging in body size (different body sizes within the same taxonomic group)
- Compare survival/body size/growth/reproduction performance of selected species in flower bulb fields affected by pesticides, grassland (nutrients) and in clean dune area
- Estimate linkage of pesticide contamination with species traits referring to reproduction strategy and body size

Methods: In situ exposure using caged organisms (4 species: *Daphnia magna, Chydorus sphaericus, Gammarus roeseli, Asellus aquaticus*). Test organisms deployed into the chambers in the field and exposed during 21 day. Scope set in controlled semi-field environment. Single stressor is tested. Many co-factors are taken into account. Estimated parameters: survival, body size, production of juveniles.

In situ testing provides advantages over laboratory toxicity testing or biosurveys. The main advantage is that more realistic exposure conditions are created allowing testing multiple stressors (mixtures of pesticides/fertilizers in flower bulb fields). In situ exposure method provides information complementing other approaches and reduces uncertainty of linking laboratory and field responses.

Research area: Same area as in previous chapter. Flower growing region in Southern Holland. Field sites selected in 3 areas: flower bulb fields, grassland and sandy dunes

Results expected:
There is difference in survival/reproduction/growth rate between flower bulb fields/pasture/dune area: reduction of survival/ growth in contaminated sites, number of juveniles produced is expected to be higher in non-contaminated sites. Reproduction performance negatively affected by pesticides.
Larger species are more sensitive to pesticides contamination because they molt more often during reproduction cycle. Smaller species reproduce faster and have shorter generation time and therefore are less vulnerable to pesticides. However smaller species have higher surface-to-volume ratio, higher absorption coefficient and therefore are more prone to pesticide contamination

Timing: March – December 2011, March – December 2012
Costs: Pesticides and nutrient measurement done in OMEGAM Laboratory
Collaboration: Water Management Board, RIVM

N	Nn	Work package/Milestones	Timing		Type of work	Collaboration	2010				2011												2012												2013												2014							
			Start	End			9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8
WP1		Literature overeview	Sept 2010	Dec 2010	Literature research																																																	
	1	Effect of chemical stressors on aquatic ecosystems (organism, species, population, ecosystem level)																																																		
	2	Risk assessment of chemicals in freshwater aquatic ecosystems. Main approaches. Methods																																																	
	3	Pesticide usage in the Netherlands																																																	
WP2		Macroinvertebrate diversity across the gradient of land use in Southern Holland	March 2011	Oct 2011		Water Managment Board																																															
	1	Literature research on macroinvertebrate communty diversity depending on land use practices and soil types																																																	
	2	Analysis of historical data on macrofauna diversity, pesticides, hydrology																																																
WP3		Impact of pesticides on taxonomic and trait composition of freshwater macrofauna	March 2011	July 2013	Field work, Lab work	RIVM, Water Managment Board																																														
	1	Literature overview on avaliable data																																																	
	2	Macrofauna sampling, water chemistry/pesticide measurements in the research area - flower bulb growing region, Southern Holland																																																	
	3	Taxonomic identification, Evaluation of selected species traits																																																	
	4	Analysis of results																																																	
WP4		Effect of pesticides mixture toxicity on reproduction and growth rate of 4 Amphipoda species	Feb 2011	Dec 2012	Field experiment	RIVM, Water Managment Board, OMEGAM Lab																																														
	1	Preparation for experiment, species culturing				RIVM																																													
	2	In situ exposure experiment with 5 Amphipoda species ranging in body size, reproduction and feeding strategy																																																	
	3	Evaluation of results																																																	
		Preparing publications																																																	
		Trainings, conferences																																																

Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Fate and toxicity of nanoparticles in ecosystems

2nd periodic report

31 March 2011

Early stage researcher:
Lan Song

Project supervisor:
Prof. Willie J.G.M. Peijnenburg
Dr. Martina G. Vijver

Research Institution:
Institute of Environmental Science, Leiden University

Executive Summary

This PhD position is sponsored by the Environmental ChemoInformatics (ECO), Marie Curie Initial Training Network within the 7th research framework programme of the European Union since August, 2010. Topic of this position is focused on investigating the fate and toxicity of nanoparticles (NPs) in ecosystems. All activities and results achieved in the second quarter of this PHD are documented in this report.

Objectives for 2nd period

In order to further investigate and develop the methodology to estimate the fate and potential risk of NPs and to protect the ecosystem as a whole, the main aim of the 2nd quarter is to focus on:

1. Literature study. Mainly focus on useful information related to experimental design.

2. Prepare for the first experiments.

3. Revise Paper: *Smart nanotoxicity testing for biodiversity conservation* (a new title of the paper mentioned as "A traits-based framework to extrapolate the toxicity of NPs within and across species" in the 1st periodic report).

4. Finish working plan for four years of PhD-project.

Activities

Activities carried out during the 2nd quarter include:

Dec. 2010: Refinement of the working plan for four years of the PhD-project.

Dec. 2010 -Feb. 2011 Preparations for the first experiment. Design of all the details of the first experiment.

Jan. -Feb. 2011: Revision of the paper "A traits-based framework to extrapolate the toxicity of NPs within and across species".

Feb. 2011: Give Presentation named "*Smart nanotoxicity testing for biodiversity conservation*" in the Institute of Environmental Science of Leiden University.

Feb. 2011: Participation in the 1st Winter School of the Marie Curie ITN programme. Activities included:

a. Training in PLS methods.

b. Advanced training in OCHEM.

c. Determination of pharmaceuticals with Liquid chromatography tandem mass spectrometry (LC-MS/MS) after solid phase extraction.

d. Scientific project management

Mar. 2011: Give Presentation named "*Smart nanotoxicity testing for biodiversity conservation*" in National Institute for Public health and the Environment (RIVM), The Netherlands.

Mar. 2011: Ordered chemicals, arranged facilities and material and cultured and keep maintaining species for experiment.

Main Achievements

The main achievements during the 1st quarter of PhD include:

1. Finished working plan for four years of PhD-project.

2. Finished the design of the first experiments.

3. Arranged all the materials and facilities for the first experiment

4. Cultured species for the first experiment.

5. Submitted Paper - A traits-based framework to extrapolate the toxicity of NPs within and across species. Submitted in Feb 2011 to the journal *Nature Nanotechnology*.

Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Project report 1 /2011

13 April 2011

LTR 7: New molecular descriptors for estimating degradation and environmental fate of organic pollutants by QSAR/QSPR models within REACH.

Early stage researcher:
Kamel Mansouri

Project supervisor:
Prof. Roberto Todeschini

Research Institution:
Milano Chemometrics and QSAR Research Group
Department of Environmental Sciences
University of Milano Bicocca. Italy

Overview:

The first two work packages (WP1: literature review and WP2: practical training) started at the same time, nevertheless during the first three months I was more focusing on the WP1 (month 1 – month 6) as described in the last quarter report which was mostly dedicated to the bibliography. After reviewing the must reads about the QSAR modelling and environmental sciences, to acquire the theoretical knowledge in the field, I started concentrating on practical training as well, as it is the goal of the WP2 during the 12 first months. For that, with the help of my supervisor and advisors, I learned using the available tools in our lab internally but also in external workshops.

Attended workshops:

The winter school in Idstein (Germany) was the second school within the ECO project. It was a very interesting one week workshop, especially the chemoinformatics course as it is perfectly in time with my scheduling of the practical training. That two days intensive theoretical and practical course in chemometrics and data mining was focusing on the most important tools of multivariate analyses using UnscramblerX software. The Principal Component Analysis (PCA) was in the main interests of the course. A great part of the course was dedicated to the multivariate regression steps of modelling, validation and prediction using different methods such as Multiple Linear Regression (MLR), Principal Component Regression (PCR) and Partial Least Squares Regression (PLSR).

The last external training I attended was the school of chemometrics and process monitoring in Modena (Italy). That three days workshop provided me with good theoretical and practical knowledge on multivariate methods such as PCA and PLS. It was also a nice opportunity to meet and interact with people from industry and have an idea about batch process methods and industrial applications.

Used Tools:

During this first period of the WP2 I was able to use some chemoinformatic tools as part of my self training. The most important ones are the OECD QSAR toolbox, the OCHEM online database and EpiSuite. These tools have in common rich databases of compounds with their 2D structures and experimental physico-chemical properties. In addition, it is possible to use them to predict a large number of endpoints with great interest to REACH using trend analysis, read across and some implemented QSAR models. With the OCHEM web-platform we can also build new QSAR models using its powerful machine learning methods, validate and make them accessible for other users.

In that training time I had also the opportunity to use the Dragon software for calculating a large number of different molecular descriptors classes. With

the help of my advisors, I was able to improve my programming skills using the powerful scientific language and software Matlab. That provided me with better knowledge of the most important steps of QSAR Modelling. It was also very useful for me to focus on deep understanding of the mostly used algorithms in the field to perform the different calculations of the variable selection, build and validate the prediction models. The knowledge of algorithms such as the genetic algorithm, support vector machines, multiple linear regression, and artificial neural networks is of prime concern for me to be able to rebuild already developed models as a preparation for the next steps.

Achieved tasks:

As sited in the last quarter report, the BioConcentration Factor (BCF) is of major concern to REACH and one of the actual studied endpoints in our laboratory. For that we started a collaboration with the research group led by Prof. E. Benfenati (Department of Environmental Chemistry and Toxicology, Institute of Pharmacological Research Mario Negri, Milan, Italy), who already worked on BCF for CAESAR project. They provided us with the dataset they used to build their five validated models. As a self training I was able to reproduce the best two models using a radial basis function neural network (RBFNN) implemented in a Matlab function that I applied on their selected descriptors values before trying to apply some feature selection methods.

In order to evaluate two new classes of molecular descriptors (i.e., spectral indices), I performed a principal component analysis on three different datasets. These families of spectral indices are based on the Eigenvalues of square matrices describing molecules in terms of different atom-atom distances or any other weighted relationships. Tested in some univariate models, these indices showed interesting property modeling ability. So the multivariate study was conducted for more understanding of the information contained in these descriptors and to make a comparison with other 2D descriptors. All descriptors calculations and PCA were performed using Dragon software.

Further work

The goal of the WP1, as an intensive literature review providing with the first theoretical knowledge in the field, was reached so far. With all internal trainings and attended meetings and workshops, the WP2 is well initiated as a preparation for me to work on it in parallel with the next work package (WP3: apply learned methods on real data) that is starting next month.

Acquired knowledge form attended and incoming courses will be used to optimally perform next tasks such as the LogP-1000 challenge consisting of building new better QSAR models to predict the partition coefficient. We are also preparing a comparison of the different applicability domain approaches. This study will be published as a poster in the next SETAC meeting in Milan.

MARIE CURIE INITIAL TRAINING NETWORK

ENVIRONMENTAL CHEMOINFORMATICS (ECO)

Project report 1 / 2011

14 April 2011

TOOLS FOR PREDICTION OF ENVIRONMENTAL PROPERTIES OF CHEMICALS BY QSAR / QSPR WITHIN REACH

(LTR-4 POSITION UNDER ECO PROJECT)

Early stage researcher:
Faizan Sahigara

Project supervisor:
Prof. Roberto Todeschini

Research Institution:
University Degli Studi di Milano Bicocca, Italy

1. *MAJOR GOALS*

The second quarter for this research mainly focussed on the following activities:

1) Thorough literature review of articles discussing already proposed Applicability Domain approaches for QSAR models.
2) Generation of MATLAB codes to implement the above mentioned Applicability Domain approaches.
3) Comparing the efficiency of these approaches by implementing them on some simulated datasets.
4) Developing a novel Centered Leverage based Applicability Domain approach proposed at our research group.

2. *TRAINING AND WORKSHOPS*

Date	Activity
21st - 25th Feb 2011	Marie Curie ECO-ITN 1st Winter School, Hochschule Fresenius, Idstein, Germany
14th - 16th Mar 2011	School on Chemometrics Methods for Process Monitoring, Modena, Italy

Regularly attended internal presentations at Environmental Sciences department of our university. This includes the work presented by other PhD students and some lectures delivered on Chemometrics and QSAR.

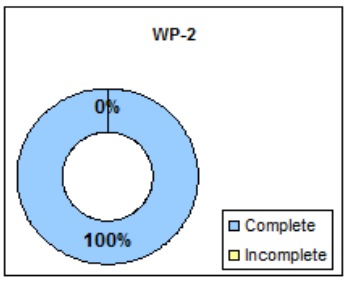
3. *WORK PACKAGES AND MILESTONES*

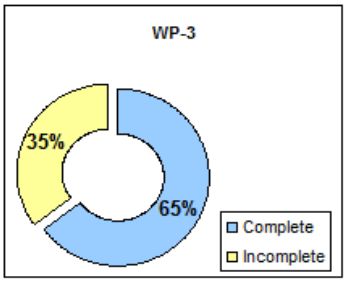
The section deals with major activities that were accomplished or initiated during this timescale and thus contributed to overall progress of the research.

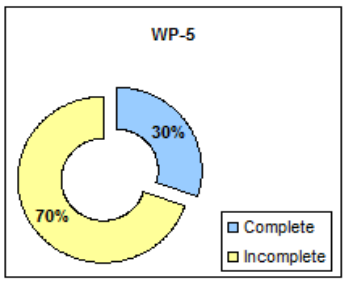
Note: According to the project plan submitted earlier, the entire research with expected duration of 3 years is divided into 8 Work Packages (WP) and 10 Milestones. The summary of all the Work Packages and Milestones can be found in the Appendix I of this report.

The second quarter of this research resumed the execution of 3 work packages that were initiated during the 1st quarter, amongst which WP-2 (Congenerity Principle and QSAR models) was successfully completed whereas WP-3 (Analysis of available approaches to define applicability domain) and WP-5 (Development of reliable QSAR models) were overlapped due to a need of simultaneous research tasks to be accomplished. All these 3 work packages progressed according to the pre-planning carried out earlier and with satisfactory results.

The tables below provide all necessary information about progress till-date for all work-packages involved during this phase.







Charts above represent the current progress of 3 work packages: WP2 (Congenerity Principle and QSAR models), WP3 (Analysis of available approaches to define applicability domain) and WP5 (Development of reliable QSAR models)

An overview of tasks accomplished:

1) Thorough literature review was carried out to understand the significance of evaluating Congenerity Principle for reliable QSAR predictions.

2) Several approaches for defining Applicability Domain (AD) has been already proposed in published articles. Research during this time-scale mainly focussed on thorough analysis of these strategies, their implementation developing Matlab codes and in future by benchmarking them on several simulated datasets.

3) Attendance to the ECO-ITN Winter School helped in further understanding of important topics like Principal Component Analysis (PCA), Multiple Linear Regression (MLR) and Partial Least Squares Regression (PLS). The school also contributed to my existing knowledge of techniques like Liquid Chromatography tandem mass spectrometry (LC-MS/MS). Also, participation in the Chemometrics School in Modena, Italy had been very beneficial in understanding the core aspects of Process Monitoring, Calibration methods, Data Pre-treatment and Regression analysis.

Major achievements:

1) Following Distanced based, Geometric, Range and Leverage based Applicability Domain approaches were documented and implemented on MATLAB. This implementation enabled a better understanding of their efficiency in defining the domain of applicability and also the significant drawbacks associated with them.

No.	Title	Category
1.	Bounding Box Approach	Range Based
2.	Bounding Box Approach with PCA Analysis	Range Based
3.	Convex Hull Approach	Geometric
4.	Classical Leverage Approach / Hotelling T2	Distance Based
5	Mahalanobis Distance based Approach	Distance Based
6	City Block Distance based Approach	Distance Based
7	Euclidian Distance based Approach	Distance Based
8	TOPKAT Optimal Prediction Space Approach	Similar to Approach No. 2, mainly differs in data pre-treatment (Centering).

2) Apart from this, I had been consistently involved in implementing the novel Applicability Domain (AD) approach that is being developed at our research group. The basis for this novel approach is Centering Leverages. For now the approach is still under execution and might expect some modifications to the current strategy for achieving the desired output.

Milestones Accomplished:

M 2.1: *Significance of Congenerity principle in QSAR.*
M 3.1: *Development of MATLAB modules to implement different approaches for defining the domain of applicability.*

Challenges:

1) Several published articles mention and discuss about various AD approaches however most of them fail to provide clear information about their implementation. This hinders the reader's task for reproducibility of these approaches and also diverts sometimes from achieving desired and satisfactory results.

2) Different Applicability Domain approaches are based on different strategies, for example, some are distance based while others are Geometric, and as a result, they have their own advantages and drawbacks associated. It is a challenging task to design a novel approach that can overcome all these limitations within a single strategy.

4. AN OVERVIEW OF FUTURE TASKS

Following summarises the research work to be carried out in the near future:

1) To continue implementing other Applicability Domain approaches and benchmarking them on several simulated datasets.

2) To further develop and implement the novel Applicability Domain approach at our research group followed by it's benchmarking.

3) The performance and output of the above implemented Applicability Domain approaches will be discussed as a poster presentation at SETAC Europe 21st Annual meeting scheduled in May 2011.

TOOLS FOR PREDICTION OF ENVIRONMENTAL PROPERTIES OF CHEMICALS BY QSAR / QSPR WITHIN REACH

An overview of research tasks and expected results

This position within the ECO project is mainly focussed on the development of new QSAR strategies to evaluate both, the congenerity principle and applicability domain. This is of major significance to develop reliable QSAR models and assist in validation process to judge whether the modelled chemical properties are predicted reliably in a given chemical space or not. Utilizing several classical and novel approaches like Multiple Linear Regression, Ranking methods and Genetic Algorithm, reliable QSAR models can be generated whereas critical evaluation and benchmarking of existing approaches to define the domain of applicability are expected to validate the model performance. The advantages and limitations of existing approaches can be beneficial to propose novel strategies that can overcome the current drawbacks if not completely, then in best possible way to achieve more reliable validation of QSAR models for chemical properties that are of high concern to REACH.

Work packages, Milestones defined for the project work and collaboration with other partner research groups under ECO Project:

Work Package 1 : **Pre-requisites to initiate the core research**		**Expected Duration:** **Sep 2010 – Oct 2010**
Aims and Objectives	Before developing any innovative ideas or strategies, it is mandatory to review the work already done within that domain of research. Also, logical planning is a must to give right direction to one's research and achieving future tasks. This work package aims to fulfil this task.	
Description of work	Retrieving relevant published articles from journals and books related to REACH endpoints, congenerity principle, development of QSAR models and defining Applicability domain. To get familiarised with available computational tools, software and servers that are needed to perform future tasks. Setting goals to achieve a smooth workflow for the entire duration of research.	
Milestones: **M 1.1:** *Literature review and efficient planning of the research work*		

Work Package 2 : **Congenerity Principle and QSAR models**		**Expected Duration:** **Nov 2010 –Mar 2011**
Aims and Objectives	To develop new QSAR strategies, evaluation of congenerity principle is a critical task. This work package aims at understanding the significance of congenerity principle for reliable QSAR predictions.	
Description of work	Review some published articles to get a better understanding of congenerity principle and its significance to generate datasets.	
Milestones: **M 2.1**: *Significance of Congenerity principle in QSAR.*		

Work Package 3 : **Analysis of available approaches to define applicability domain.**	**Expected Duration:** **Oct 2010–Jun 2011**
Aims and Objectives	There are several approaches already developed till date to define applicability domain for QSAR models, based on structural aspects, physicochemical response or mechanistic understanding, however most of them offer several advantages accompanied with some major limitations. This work package is aimed at comparing the performance of these approaches by subjecting them to some simulated QSAR datasets.
Description of work	Using several simulated datasets, the performance check will be carried out to see how efficiently different approaches can predict the domain applicability. The advantages and disadvantages associated with each approach will be documented.
Milestones: **M 3.1**: *Development of MATLAB modules to implement different approaches for defining the domain of applicability.* **M 3.2** : *Benchmarking of existing applicability domain approaches.* **M 3.3** : *Comparison the results for different approaches on simulated or published models.*	

Work Package 4 : **Propose novel approaches for defining the domain of applicability**	**Expected Duration:** **Jun 2011 – Dec 2011**
Aims and Objectives	This work package deal with addressing the current drawbacks that needs to be considered in the future. Main motto however will be to propose some new approaches for defining the applicability domain in order to eradicate in a best possible way the prevailing drawbacks.
Description of work	The initial focus will be on the potential of available approaches to reliably predict the applicability domain of QSAR models and the drawbacks can be considered as the starting point for proposing some new approaches. The idea behind developing such new strategies will be to overcome the current pitfalls, if not completely then in the best possible way.
Milestones: **M 4.1:** *Selection of appropriate algorithms and strategies to overcome the existing pitfalls in current applicability domain approaches.*	

Work Package 5 : **Development of reliable QSAR models**	**Expected Duration:** **Dec 2010- Jan 2012**
Aims and Objectives	This work package is crucial in order to develop some reliable QSAR models. As the research is contributing to REACH, this task demands careful selection of endpoints that are of high concern and building datasets with a better choice of chemical structures.
Description of work	Reviewing REACH regulations to select the endpoints of high concern. Using available molecular modelling packages to build chemical structures which are then added to the training set. Generate training sets for the selected endpoints. Finally, to get some QSAR models with best possible prediction and thus, higher reliability.
Milestones: **M 5.1:** *Selection of endpoints relevant to REACH, generate datasets and build reliable QSAR models.*	

Work Package 6 : **Validation of the developed QSAR models**	**Expected Duration:** **Dec 2011- July 2012**
Aims and Objectives	QSAR validation is a critical phase to analyse performance of the models and interpret their mechanistic relevance. The third principle for OECD guidance on model validation requires defining a domain of applicability for QSAR models to make a performance check. This work package is mainly dedicated to the better understanding of applicability domain, considering approaches already proposed or published and reviewing their advantages and limitations.
Description of work	Review of OECD guidelines for QSAR validation with main focus on applicability domain. Detailed review of approaches already developed to define applicability domain.
Milestones: **M 6.1:** *Critical analysis of current approaches to define applicability domain.*	

Work Package 7 : **Practical implementation of the proposed novel approaches**	**Expected Duration:** **Jan 2012- Mar 2013**
Aims and Objectives	*'Is it feasible to practically implement the proposed approaches?'* The package will be mainly aimed at putting efforts to make it possible implementing the proposed approaches and providing their mechanistic relevance, if possible.
Description of work	Think about possible ways to test the novel approaches on real QSAR data. Evaluating prediction quality and reliable interpretation by performing the validation check on these novel approaches.
Milestones: **M 7.1**: *Evaluation of proposed approaches on several real QSAR datasets (generated in work package 5 and 6).*	

Work Package 8 : **Thesis preparation**	**Expected Duration:** **Mar 2013- Aug 2013**
Aims and Objectives	The last package is contributed to the thesis writing, preparation for exam and presentation of research work to the examiners for their feedback and opinions.
Description of work	Documenting the methodologies, results and conclusion of the work conducted in the entire duration of this research.
Milestones: **M 8.1:** *Thesis writing and presentation of research work to the exam committee.*	

Collaboration with other partner research groups under ECO project		
No	**Participating research group**	**Tasks**
1	Helmholtz Zentrum München, Germany	i) Testing of applicability domain ii) Speciation
2	Hoegskolan i Kalmar, Sweden	Use of chemical categories

Marie Curie Initial Training Network

Environmental Chemoinformatics (ITN-ECO)

Project report

20th March 2011

The potential of cell-based *in vitro* assays for studying toxic effects of chemicals

Early stage researcher:
Tobias Lammel

Project supervisor:
Dr. José M. Navas

Research Institution:
Instituto Nacional de Investigación y Technología Agraria y Alimentaria (INIA)

A. Introduction

The first months of the project were dedicated (besides literature research) to study a particular mechanism of toxic action. This report will give a short introduction to the scientific background of the research question that was addressed within the first nine months of the thesis. Furthermore it will give a summary over the results obtained to this day.

B. Project report

1. Scientific background

The activation of the AhR-mediated pathway is known to elicit numerous toxic responses. Of particular concern is its cross-talk with other intracellular pathways leading to endocrine disruption. Thus, understanding the mechanisms that underlie AhR activation is of outmost interest for the correct evaluation of the toxic potential of chemicals. The research carried out within the first months of work aimed to investigate the interaction of imidazole fungicides with the Aryl hydrocarbon receptor (AhR).

The classical mechanism of AhR activation is binding of an agonist to an appropriate site on the receptor. High-affinity ligands share a number of structural characteristics. They are all polycyclic aromatic hydrocarbons having at least two of their rings in the same plane. However, the AhR can also be activated by compounds with structures dramatically different from that of prototypical ligands. It has been suggested that binding of these compounds might occur to a second, rather promiscuous ligand-binding site on the receptor. Some compounds are able to activate the AhR indirectly, probably via alternative intracellular signal pathways involving phosphorylation events, demonstrating that ligand-binding is not the solely mechanism of AhR activation.

2. Research objective

The objective of our current research is to elucidate the mechanism through which the imidazole fungizide Prochloraz activates the AhR and to find out (at a later date) which aspects have to be in particular considered when the toxic potential of compounds like Prochloraz (weak CYP1A inducers, not able to directly bind to the AhR) are evaluated by means of cell-based in vitro assays.

3. Results and Discussion

Cytotoxicity of Prochloraz

The cytotoxicity of PRO towards the rainbow trout cell line RTG-2 was determined by means of two cytotoxicity assays (MTT-Assay, Neutral Red Retention-Assay). PRO was shown to cause cytotoxicity at concentrations of 80 µM (LOEC) after 24 hrs and 48 hrs. After 72 hrs of exposure the lowest concentration at which an effect in comparison to the control could be detected was 10 µM.

Induction of CYP1A

PRO was shown to increase AhR-mediated transcription of *cytochromeP450 1A (CYP1A)* in RTG-2 cells. Furthermore, elevated levels of CYP1A-dependent ethoxyresorufin-*O*-deethylase (EROD) activity demonstrated the induction of CYP1A at the protein level. In both cases, the PRO-dependent CYP1A induction was found to be much lower than that measured in cells, which were exposed to the AhR model agonist β-Naphtoflavone (β-NF).

Computational modelling of the molecule structure

Interestingly, computational modelling of the lowest energy conformation of PRO revealed that both aromatic moieties of the molecule (the imidazole and the benzene ring) are highly unlikely to lie in the same plane. This suggests that PRO is not able to activate the AhR via ligand-binding.

Interaction of PRO with the AhR

The hypothesis that PRO is not a ligand of the AhR was tested by means of a commercially available kit (AhR-Immunoassay®), which allows to determine whether a given compound binds to the AhR. In this assay PRO was shown not to bind to the receptor. Therefore, the question arose through which mechanism, if not ligand-binding, the imidazole fungizide activates the AhR.

Interaction of a metabolite of PRO with the AhR

Based on the above mentioned as well as previous results (experiments using protein kinase inhibitors did not give any evidence that alternative signalling pathways may be involved in the activation of the AhR) we hypothesized that not the parent compound, but one of its metabolites activates the receptor.

Therefore, it was tested whether the inhibition of xenobiotic metabolism using the irreversible cytochrome-P450 inhibitor 1-Aminobenzotriazole (ABT) results in a decrease of PRO induced CYP1A expression levels. RTG-2 cells cultured in 6-well plates were pre-exposed to ABT for 2hrs by replacing the used culture media with ABT containing culture media. Subsequently, the ABT containing media was removed and replaced by media containing both, the inhibitor and PRO. Following total RNA extraction CYP1A mRNA levels were semi-quantified by means of RT-PCR. Unexpectedly, CYP1A mRNA levels in cells co-exposed to ABT and PRO seemed to be higher than in cells that were only exposed to PRO. This observation lead to the suggestion that either ABT or a breakdown product of one of the compounds included in the cell culture media (e.g. Tryptophan) is also able to induce CYP1A.

Indeed, experiments conducted in consequence revealed that solely replacing the spent media with fresh media already leads to an increase in CYP1A expression. Moreover, ABT was found to be an inducer of CYP1A as well.

Thus, further experiments were carried out in order to identify the optimal concentration and exposure time for ABT (i.e. the time and concentration that results in a sufficient inhibition of xenobiotic metabolism and keep the induction of CYP1A on a minimum).

Additionally, the protocol was modified in that way that a media change could be avoided. Instead of replacing the whole cell culture media, smaller volumes of a higher concentrated solution of the inhibitor and/or the test compound were added. Currently it is investigated whether these as optimal identified exposure conditions allow us to demonstrate the implication of a metabolite of PRO in the activation of the AhR.

Identification of potential metabolites

In parallel, in cooperation with the Institute for Organic Chemistry (CSIC), we aim to obtain a profile of the metabolites of PRO that are formed in vitro. Therefore, PRO will be incubated with S9 fractions prepared from RTG-2 cells, the metabolic reactions terminated after different times points, the organic compounds extracted from the sample, separated by means of HPLC and thereupon analysed and tried to be identified using mass spectrometry.

C. Perspective

First results will be expected by June 2011. If they are in accordance with the working hypothesis (AhR is activated by a metabolite of Prochloraz) a first paper will be written and submitted to an appropriate journal.

Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Project report 1/2011

31 March 2011

General mechanisms in biotransformation of chemicals: Linking Michaelis (K_m, V_{max}) constants to lipophilicity

Early stage researcher:
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Project supervisor:
A. Jan Hendriks

Research Institution:
Radboud University Nijmegen, The Netherlands

Working package: General mechanisms in biotransformation of chemicals: Linking Michaelis (K_m, V_{max}) constants to lipophilicity (WP1).

Purpose of the working package: The aim of WP1 is the prediction of K_m and V_{max} for different chemicals based on lipophilicity, thus gaining insight into the general mechanisms of biotransformation.

Background information: REACH legislation encourages the use of models for the risk assessment of chemicals. In bioaccumulation models, biotransformation is one of the processes which decrease the concentration of metabolizable compounds in an organism, together with elimination through other physicochemical processes (e.g. elimination to water, egestion, growth[1]).
Though biotransformation rates apply to a specific combination of a chemical and a species, some general patterns are noted. For instance, accumulation of metabolizable compounds appears to be a factor of about 50 lower than that of persistent equivalents[2]. This suggests that the underlying mechanisms may be (somewhat) more universal than usually thought. Perhaps, biotransformation potential of xenobiotic as well as biotic compounds has evolved according to similar principles. So far, such general mechanisms have hardly been investigated.

Description of accomplishment: Task 1 (literature research) was completed, in accordance with the working plan. Information on turnover of biotic compounds and on biotransformation of xenobiotics was collected and studied for the settlement of the thesis plan. The literature research brought out two main subjects:

1. Need to further develop and evaluate the methods for estimating biotransformation rates (k_m).

The accumulation of xenobiotics in organisms depends on transport and transformation processes. While rates of exchange with air, water, and food can be predicted from properties of chemicals and biological species[1, 3], biotransformation rates are difficult to obtain. Recently, *in silico* models have been developed to derive k_m values of chemicals with QSARs for fish[4] or by comparing accumulation and elimination of stable and labile compounds for various biological species[5]. Methods were also proposed to extrapolate *in vitro* measures to whole body k_m values in fish[6].

2. Compound lipophilicity may play a major role in binding affinity to enzymes.

The enzymatic action of metabolism involves two processes: first, the chemical must reach the enzyme and bind to it; second, a catalytic reaction must take place. The binding of the chemical and its successive catalysis are described by two enzymatic parameters: the Michaelis constant (K_m) and the maximum rate of the reaction (V_{max}), respectively[7]. Any enzymatic reaction is characterized by its value of K_m, which is the substrate concentration at half V_{max} and is independent of the enzyme concentration[8].
QSARs have been developed to relate metabolic parameters and chemical characteristics, mainly focusing on *in vitro* pharmaceutical metabolism mediated by cytochrome P450[9-10]. It was shown that substrate binding affinities obtained from K_m data exhibit linear correlations with lipophilicity (expressed as K_{ow}). Therefore, it can be assumed that compound lipophilicity plays a major role in biotransformation of xenobiotics and this has an effect on the overall clearance of such compounds. Other factors might also be involved in enzyme binding, such as polarity, hydrogen bond donor and/or acceptor properties[11-12].

On the other hand, the catalytic process (represented by V_{max}) resulted to be less controlled by substrate lipophilicity and more influenced by electronic properties of the substrates, such as electrophilicity[7].

Significance of accomplishment: Following the literature research, the relationships between Michaelis parameters (K_m and V_{max}) and lipophilicity (K_{ow}) were chosen as a preliminary method to investigate the possible underlying mechanisms of metabolism. The relationships between metabolic constants and other substrate properties will be analyzed in WP2.
In the present working package, the relationships are applied to enzymes involved in the metabolism of groups of chemicals in different species. The overview of these relationships should provide some information about general patterns of metabolism, which could then be applied in risk assessment.

Current status: Task 2 (data collection) and Task 3 (model development) are ongoing, in accordance with the working plan.
Experimental K_m and V_{max} values (*in vitro*) for different enzymes are currently being collected, and the relationships of these data with compound lipophilicity are being processed. The resulting QSARs, built merging all the compounds metabolized by one enzyme family, will be compared in order to find general patterns for different chemicals (e.g. endobiotics and xenobiotics, drugs and pesticides) and for different species (e.g. mammals and fish).

Problems: None.

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Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Project report 1 /2011

13 April 2011

Report for the reviewers

Early stage researcher:
Isabel A. O`Connor

Project supervisor:
A. Jan Hendriks

Research Institution:
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Milestone 1: Inclusion of new descriptors into bioaccumulation models

Improving the prediction of uptake of polar compounds[1] via passive diffusion by either incorporating additional descriptors into existing models such as OMEGA [1], or developing a new model.

Background

In the EU, REACH requires the assessment of compounds regarding their potential to accumulate and induce toxic effects in organisms in the environment. Since it is not possible to test all chemicals, models are needed to predict bioaccumulation and toxicity of these untested compounds. Mechanistic bioaccumulation models often include the following processes: uptake and elimination by absorption through gills/lungs/skin, by food through the gastro intestinal tract (GIT), and elimination by transformation and growth (see e.g. [1], [2]). During uptake processes, the chemical has to permeate through several lipid and aqueous layers, which are considered as barriers with respective resistances [1, 3]. The resistance of the membrane layers depends on the lipophilicity of the chemical. The octanol water partition coefficient (K_{ow}) is a good indicator for the compound lipophilicity and is commonly included in bioaccumulation models. However, for polar compounds K_{ow} is not sufficient. Therefore, the first milestone is to improve the prediction of uptake of polar compounds via passive diffusion by introducing new descriptors. Carrier mediated transport will be included in the next project (milestone 2).

WP 1: Literature research

Status: Completed as the main task, in accordance with the work plan.

Results: During literature research knowledge was gained about mechanisms of permeation through membranes, why K_{ow} is not a sufficient descriptor and how permeation has been modeled so far. In pharmaceutical studies, descriptors accounting for polar interactions such as hydrogen bond donor and acceptor, polar surface area, etc, were widely used to predict human oral absorption of pharmaceuticals (see overview in [4]). Usually, these descriptors were included into linear and nonlinear multi regressions models (see e.g. [5], [6], [7]), or suggested as cut off values ([8], [9]). Environmental bioaccumulation models focused up to now mainly on very hydrophilic compounds and only few approaches included or looked at polar descriptors in bioaccumulation models (see e.g. [10-11]).

WP 2: Data collection

Status: Completed, faster than expected.

Results: Uptake rate data [L kg^{-1} d^{-1}], faction absorbed [%] and BCF [-] of polar compounds were collected for animals. During data mining also the data set of Zhao et al [7] was found, which describes human oral absorption of pharmaceuticals.

[1] Polar compounds are here defined as compounds with a $\log K_{ow} < 3$ and at least 1 N or O

WP 3: Modeling

Status: Ongoing, in accordance with the work plan.

Results: The data set for animals was problematic and difficult to model. However, a new model was developed for Zhao`s data set: The OMEGA model [1] for prediction of uptake from water through gills was adapted to describe uptake from the GIT by drinking contaminated water. Abrahams hydrogen bond donor descriptor A [12] was included in this drinking water model and the model was fitted to the data set. The new model (RMSE=14.54, r^2= 0.73) described the data better than the drinking water model, which was based on K_{ow} only (RMSE=81,32, r^2 = 0.64). The new model predicted absorption based on K_{ow}, but now absorption for compounds with high A were corrected towards lower absorption, thus following mechanistic reasoning. Zhao's model obtained similar statistics, but it was based on nonlinear multivariate regression analysis and provided less mechanistic explanation than the new model.

During this work a second hypothesis was developed: Sorption to food lipids may not be relevant and thus can be neglected for oral uptake of polar compounds. Therefore, the drinking water model can be merged with the food uptake model for polar compounds. The aim is to create one model valid for the logK_{ow} range from -10 to +10, with supporting data for the whole K_{ow} range. This work is still in progress.

Next steps

The next step will be to merge the OMEGA "drinking water" and "food absorption" models in order to obtain one model for the whole K_{ow} range. If this works out, Abraham's hydrogen bond donor descriptor A will be included in this merged model.

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Marie Curie Initial Training Network

Environmental Chemoinformatics (ECO)

Project report 1 /2011

13 April 2011

In Vitro Screening of Fullerene (C_{60}): pure and in combination with xenobiotic organic compounds

Early stage researcher:
RAJESH RATHORE

Project supervisor:
Prof. Dr. K.-W. Schramm

Research Institution:
IOEC, HelmholtzZentrum Muenchen

THIRD QUARTER REPORT

A SHORT SUMMARY OF LAST QUARTER REPORTS

1. Literature search
2. Handling of *Tetrahymena thermophila* and Rat Hepatoma Cell (*H4IIE*) culture
3. Preliminary testing of Printex® 90 and TiO_2 nanoparticles with protozoa
4. Preparation of fullerene (C_{60}) and its preliminary testing with H4IIE cell line.
5. Attended one conference and two workshops.

Work Packages

1. To measure the influence of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in presence of Aqu nC_{60} on Micro-EROD-Assay (*in vitro*)

Background

Aromatic hydrocarbons such as 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) are able to pass through biological membranes specific binding to the aryl hydrocarbon receptor (AhR). This is known to participate in multiple mechanisms of the normal physiology in vertebrates being able to generate biological and toxicological effects. The AhR mediates the induction of specific enzymes, in particular, the cytochrome P450 1A1 (CYP 1A1). By means of this bioassay, the induction of CYP 1A1 can be determined as the sum of the whole compounds able to elicit a response in a determined sample dioxin in presence of fullerene.

Procedure

The micro-EROD-assay is performed according to Donato et al. (1993) with some modifications. The cells were seeded with a density of 1 x 10^4/well (100 µL). After 24 hours incubation, the DMEM medium is removed and 100 µL new medium is given to the well cavities. This medium contains TCDD generating the following concentrations in the bioassay wells: 0.4 pg = 1.24 fmol; 0.2 pg = 0.62 fmol; 0.15 pg = 0.47 fmol; 0.1 pg = 0.31 fmol; 0.06 pg = 0.19 fmol; 0.02 pg = 0.06 fmol; 0.015 pg = 0.004 fmol. The TCDD standards used to perform the TCDD medium dilutions are dissolved in a solvent mixture DMSO/isopropanol 4:1; v/v. TCDD standard solutions are calculated in order to obtain a final ratio of solvent mixture/medium in the well of 0.5 % and the final well concentrations mentioned above.

Preparation for the incubation

a.) Samples and standards are stored at 4°C. 20 minutes before starting, take them from the fridge in order to achieve room temperature

The TCDD standard solutions are:

30 pg/mL, 40 pg/mL, 120 pg/mL, 200 pg/mL, 300 pg/mL, 400 pg/mL, and 800 pg/mL. A blank that consists on a solution DMSO/Isopropanol 4:1 (v/v) is included as an internal quality control.

b.) DMEM medium is tempered in a water bath at 37 °C for 10 minutes.

c.) Turn on the sterile bank 20 minutes before starting the work activities. Clean the sterile bank surfaces with ethanol 70 %.

d.) All elements within the sterile bank are also disinfected with ethanol 70 %.

Preparation of sample

a.) Preparation of Aqu-nC_{60} and its Blank

Aqueous nC_{60} solutions were prepared according to the procedure proposed by chen et al (Environ. Toxicol. Chem. 27, 2008) with slight modification. Specifically, C_{60} fullerene powders (20 mg) were allowed to dissolve completely into 20 ml of toluene using a magnetic stirrer. This solution was referred to as the C_{60}-toluene stock solution. The purple C_{60}-toluene stock solution was transferred to a beaker containing 50 ml ultrapure water and 1.5 ml ethanol. The mixture then was sonicated using a dipprobe sonicator (Bandline SONOPULS GM 70) operated until the toluene phase disappeared. The resulting yellowish/ brown suspension was filtered through a 0.7-µm glass fiber.
Blank Aqu-nC_{60} (Treated water) was prepared as mentioned above except addition of fullerene (C_{60}).

Aqu-nC_{60}

b.) Addition of TCDD to Aqu-nC_{60}

In 50 ml scintillation vials 10 ml of Aqu-nC_{60}or Treated water was added and to this 200 µL of 50 ng/L TCDD standard was added with the help of Hemilton syringe. The resulting solution was sonicated for 3-4 min. And different dilutions were made using their own blank.

Incubation

a.) In 14 mL sterile polyethylene tubes 3980 µL DMEM culture medium were added. 8 tubes are needed for the standard curve (7 standards + blank) and extra tubes for the samples.

b.) 20 µl of standard were given to the 2980 µl DMEM. 40 µl of samples (Aqu-nC_{60}or Blank Aqu-nC_{60}) are given to the 2960 µl DMEM. Each tube was thoroughly shaken to assure complete homogenization (manually or vortex).

c.) The new solutions were given to the canalized mixing chamber keeping the order such as the given in the 96 well plate allocation (see 96 well plate below).

d.) The plates were taken from the incubator and after controlling the cell growth under the microscope (about 70- 80 % confluence) the old medium is removed. After this, 100 µl of the new solutions (blank, standards, and samples) are given to the plate with a multi channel pipette.

After 24 and 72 hours exposure the incubation medium was removed. Following this, 100 µl of new DMEM medium containing a final concentration of 7-Ethoxyresorufin 8 µM and dicumarol 10 µM is added per well. This reaction mixture was prepared mixing 11640 (970) µl DMEM + 240 (200) µl 7-Ethoxyresorufin standard solution + 120 (100) µl dicumarol standard solution (mixture calculated for one 96 well plate 12 ml solution). After 30 minutes incubation at 37 °C without plate cover, the 100 µl reagent solutions were trespassed to other 96 well plate which has already 200 µl ethanol per well. Cover the plate with a lid protected with aluminium paper in order to avoid the resorufin excitation due to the light. Shake the well plate for 2-3 minutes to homogenize the mixture medium/ethanol in an orbital shaker (Heidolph, Titrimax 100; Step 4). After this, the fluorescence was measured at 535 nm excitation and 590 nm emission. To achieve the cell fixing after fluorescence measurement 50 µL glutaraldehyde 1.2 % (stored under Argon atmosphere at 2-3° C) is added per well and left for 5 minutes. Then, each well is washed with 200 µl PBS twice.

	1 Standard	2 Standard	3 Standard	4 Standard	5 Sample	6 Sample	7 Sample	8 Sample	9 Sample	10 Sample	11 Sample	12 Sample
A	BK	BK	BK	BK	BK	BK	BK	BK	BK	BK	BK	BK
B	30 pg/ml	0.015 pg/well	0.015 pg/well	0.015 pg/well								
C	40 pg/ml	0.02 pg/well	0.02 pg/well	0.02 pg/well								
D	120 pg/ml	0.06 pg/well	0.06 pg/well	0.06 pg/well	2960 µL DMEM+40 µL Aqu-nC_{60} with TCDD				2960 µL DMEM+40 µL Treated water with TCDD			
E	200 pg/ml	0.1 pg/well	0.1 pg/well	0.1 pg/well								
F	300 pg/ml	0.15 pg/well	0.15 pg/well	0.15 pg/well								
G	400 pg/ml	0.2 pg/well	0.2 pg/well	0.2 pg/well								
H	800 pg/ml	0.4 pg/well	0.4 pg/well	0.4 pg/well								

Result: Presence of aqueous fullerene nanoparticles (aqu-nc_{60}) do not alter ethoxyresorufin o-deethylase (EROD) activity induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

2. Toxicity assesment of C_{60}, $C_{60}(OH)_{24}$ and Printex®90 nanoparticles to ciliated protozoa *Tetrahymena thermophila*.

Procedure

Cell Culture

The experimental stock culture was prepared for every week from long term stock culture. This culture was reared in PPY-medium and serve as starter culture for the preparation of logarithmically growing pre-culture. 200-300 µL cell aliquot was transferred from the vial by piercing of septum with a sterile syringe and added into 40 mL PPY medium in a 300 mL Erlenmeyer flask with fine porous silicon gum head. The porous cap of the flask allows an adequate oxygen supply. Temperature of the culture flask was maintained to 28°C as same for chemical toxicity studies too.

Nanoparticles, reference compounds and exposure of *T. Thermophila*

500 µl of the toxicants (Aqu-nC_{60} $C_{60}(OH)_{24}$ and Printex®90) , in Osterhout's medium was pipetted into the wells of 24-well polystyrene culture plates (Falcon), each concentration in two replicates, and 500 µl of *T. thermophila* cells in Osterhout's medium (10^6 cells/ml) was added to the wells (final cell density in the test medium was 5×10^5 cells/ml). Osterhout's medium served as a control. In addition, a cell-free control was made, where 500µl of Osterhout's medium was added to 500 µl of toxicant suspension/solution. The test plates with protozoa were incubated for 6, 24 and 48 h at 25 ◦C in the dark, without shaking. The pH of *T. thermophila* control culture in Osterhout's medium was 6.5.

Cell viability Assay

After 6, 24 and 48 h of incubation of *T. thermophila* with or without toxicants, 100 µl was transferred from each well to 96-well black polypropylene microplate (Greiner Bio-One, Germany) for the viability testing with the fluorescent dye propidium iodide (PI, Fluka). The stock solution of PI was prepared in deionised water at a concentration of 1 mg/ml. This was further diluted with deionised water to obtain the working solution of 100 µg/ml, which was 10 times the final concentration in the viability assay. 10µl of the PI working solution was pipetted directly into each well of 96-well microplate containing 100µl of exposure medium and the microplates were further incubated for 15min at 25 ◦C in the dark. The fluorescence was quantified using the Spectra Flour

microplate reader (SLT- Labinstrument GmbH, Salzburg, Austria) at excitation and emission wavelengths of 530 and 590 nm, respectively.

Result: Study under process and modification.

2. Influence of fullerene on homeostasis of *Tetrahymena thermophila*

(Study under process)

Milestones

1. Comprehension of current state of the art on fullerene and its derivative, and their toxicity assessment.
2. Abstract for oral presentation submitted and accepted at ISTA15, Hong Kong.
3. Attended first Winter School at Hochschule Fresenius in Idstein, Germany (21-25 FEB 2011)