MistraPharma
Annual report 2010

Annual report for MistraPharma 2010

MistraPharma has now been running for three years. The activity in the program is high and data are being generated at a steady pace. Our work with identifying pharmaceuticals of environmental concern has continued during 2010. The MistraPharma prioritization process is still based on the fish plasma model, but the results obtained from this theoretical model have been followed up by two experimental bioconcentration studies. The first bioconcentration study received a lot of attention when it was published. The joint Gothenburg-Umeå press release about this study was for instance the most downloaded news at the University of Gothenburg in the spring of 2010.

The analyses of samples from the second bioconcentration study are now being finalized. Of the 90 pharmaceuticals that have been analyzed so far, 67 were detected in the water samples and 15 were detected in the fish plasma. The analyses of an additional 30 pharmaceutical substances are ongoing. The MistraPharma selection of pharmaceuticals prioritized for further testing within the program will be based on the results of these two bioconcentration studies.

Another broad prioritization approach that the MistraPharma researchers in Umeå and Gothenburg have developed is the "Critical Environmental Concentration" (CEC). Critical environmental concentrations have been calculated for 500 pharmaceuticals. The CEC value represents the (theoretical) water concentration that would cause a pharmacological response in exposed fish. A CEC value for a specific pharmaceutical can thus be directly compared to any measured concentration of that pharmaceutical in surface waters to provide a first estimate of risk.

A recent important finding from the MistraPharma team in Uppsala is that the progestin levonorgestrel, a component in contraceptives, is a potent and efficient developmental toxicant in female frogs (Kvarnryd et al. 2011a, Aquatic Toxicol.). In this study, exposure of frogs to levonorgestrel during the tadpole stage caused severe effects on the development of the female reproductive system; Egg development was interrupted and a complete lack of developed oviducts was observed. As a consequence, the females were sterile. The lowest dose tested in this experiment is approaching environmentally measured concentrations of levonorgestrel. Studies are in progress to define a no-effect-concentration, and to relate this to those present in effluent wastewaters in Sweden.

Another question that has been explored is how to facilitate the use of non-standard ecotoxicity data in environmental risk assessments of pharmaceuticals. In an empirical study, four different evaluation methods, previously proposed in the scientific literature, were used to evaluate nine non-standard ecotoxicological studies. The different evaluation methods came to surprisingly divergent results about the reliability of these studies, and the reliability of the data was generally considered low. A general conclusion from this effort is that the reporting of non-standard studies need to be significantly improved in order to facilitate their use for environmental risk assessment.

The MistraPharma database – WikiPharma – has been recently updated. The update shows that the number of ecotoxicological studies for pharmaceuticals is increasing; One year ago WikiPharma contained data for 130 pharmaceuticals. The current number is 143. During the same time period the number of references in the database has increased from 172 to 209. Feedback from researchers and regulators from several countries in Europe and North America indicate that the database is considered a useful tool in the work with environmental risk assessments of pharmaceuticals.

Analyses of the data compiled in the WikiPharma database are currently being performed with the aim to compare the sensitivity of standard and non-standard tests for identifying pharmaceuticals of ecotoxicological concern. Preliminary results show considerable differences in the sensitivity between standard and non-standard tests for a significant number of compounds. The analyses will now be supplemented with information about the pharmaceuticals' expected mode-of-action and an assessment of whether the observed differences in test sensitivity depend on the specificity of the effects in relation to the measured endpoints.

An important aim for MistraPharma is to strengthen the network between researchers and stakeholders working with environmental risks and pharmaceuticals. A major activity towards this aim is the continuous communication with the program's dedicated and knowledgeable reference group. MistraPharma's reference group currently consists of 18 representatives from our most important stakeholders. The reference group is a vital component in the work to ensure that our research will come to practical use and benefit society. To emphasize the importance of our stakeholders, the MistraPharma 2010 yearly report is focused on highlighting their crucial contributions to the collaborative process towards a non-toxic environment.

MistraPharma Board/ Christina Rudén, programme director

Content

Programme structure

Programme Board

Chair:
Ethel Forsberg, Swedish Chemicals Agency (until July)
Charlotte Unger, the Medical Products Agency (appointed from August)

Other members:
Berit Balfors, KTH
Åke Bergman, Stockholm University
Nina Cromnier, Swedish Chemicals Agency
Bengt Mattson, LIF
Lena Söderberg, The Swedish Water & Wastewater Association
Åke Wennmalm, Stockholm County Council

Co-opted members:
Britt-Marie Bertilsson, Mistra (until Dec 2010)
Christopher Folkeson Welch (from Jan 2011)
Karin Liljelund, Stella Futura
Christina Rudén, KTH

The board has held three recorded meetings during the period (100318, 100527, 101028).

Programme director

Christina Rudén

Commmunication manager

Karin Liljelund

Reference group

Berndt Björlenius, Stockholm Water
Annika Christensson, Blekinge County Council
Anders Finnson, The Swedish Water & Wastewater Association
Linda Gårdstam, Swedish Environmental Protection Agency
Britta Hedlund, Swedish Environmental Protection Agency
Gisela Holm, LIF / AstraZeneca
Nils-Gunnar Lindqvist, Swedish Chemicals Agency
Lars Lööf, Västmanland County Council
Inger Näsman, Kronans Droghandel / Swedish Pharmacy Association
Theres Olsen, Uppsala County Council
Nicklas Paxéus, Gryaab AB
Stephan Quittenbaum, Kronoberg County Council
Per Rosander, ChemSec
Lennart Sorby, Water Authority for the Northern Baltic Sea
Cajsa Wahlberg, Stockholm Water
Per Ola Darnerud, National Food Administration

Webpage and contact

www.mistrapharma.se

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Financial Report

	Outcome 2010	Outcome 2008-2010
REVENUES		
Allocated funding from Mistra	10 277 540	32 363 800
Other revenues*	406 076	585 060
TOTAL REVENUES	**10 683 076**	**32 948 860**
COST		
Personnel costs	5 713 781	16 332 455
Travel expenses	335 871	812 354
Supplies	885 064	2 265 791
Depreciation	235 672	405 490
Other operating expenses	460 707	1 561 060
DIRECT COSTS	**7 631 095**	**21 377 149**
Overhead including premises costs**	2 300 825	6 618 636
Costs including overhead	**9 931 920**	**27 995 785**
Purchased services***	173 113	345 058
University VAT****	0	576 016
Provided grants for DTU Denmark	427 179	1 205 469
TOTAL COST	**10 532 212**	**30 122 328**
BALANCE	**150 864**	**2 826 532**
ALLOCATED FUNDS PHASE 1 2008-2011		**42 060 280**
REMAINING FUNDS 2011		**12 373 012**

* LIF (Goodpoint AB), interest income, cofinancing, invoiced
** Can not exceed 35%
*** May not be debited overhead
**** 8% VAT deduction for University, year 2008

Project reports

Umeå University

Project leader: Mats Tysklind

Summary of completed research within MistraPharma

Quantify internal drug exposure

Identifying API's of high concern has continued during 2010. This on-going work is still based on the fish plasma model, i.e. calculations based on the human therapeutic plasma concentration, usage statistics (PEC) and theoretical bioconcentration, which yields an effect ratio (ER). As a complement to this approach, a multidimensional chemical map of APIs has been constructed, using principal component analysis, covering 899 drugs described by 67 calculated chemical descriptors. This "chemical map" of APIs has been applied to search for potential environmentally persistent APIs using a structural similarity tool. The basis for the tool is a selection of so-called anchor molecules (diclofenac, trimethoprim, and carbamazepine) representing APIs of known environmental concern.These results have recently been submitted.

In addition to the effect ratio (ER), which is calculated based on Swedish sales, a critical environmental concentration (CEC) was calculated for 500 APIs. This CEC value represents the (theoretical) water concentration that would cause a pharmacological response in exposed fish. These CEC values are individually calculated for each API and are based on the human therapeutic plasma concentration and theoretical bioconcentration. The benefit with this approach is that it is valid globally; any measured surface water concentration can be compared to the CEC value, which makes this a suitable early tiered approach to estimate risks. These results were presented at the SETAC conference in Seville in 2010 and are now published in Regulatory Toxicology and Pharmacology.

Our bioconcentration study of fish exposed to sewage effluent was published in Environmental Science & Technology and received a lot of attention. Our joint Gothenburg-Umeå press release about this article was e.g. the most downloaded news at the University of Gothenburg during the spring 2010.

Our follow-up study that was performed in 2009 at Gryab (Gothenburg) and downstream the sewage treatment plant in Skövde has now been analyzed. Of the 90 APIs that have been analyzed so far, 67 were detected in the water samples and 15 were detected in the fish plasma. Average concentration in the water samples was 259 ng/L and in 0.79 ng/ML in the fish plasma. This study will be concluded this spring by the analysis of the additional 30 APIs. All APIs that have been detected in fish plasma in these field studies (2008 and 2009) have been added to a prioritized list for further biological evaluation. In the 2009 study, samples were also taken of muscle, brain, liver and bile. Additional method development based on pressurized liquid extraction (PLE) is under way and these samples will be analyzed during spring 2011. This approach will make it possible to study in detail the distribution of drugs in individual exposed fish.

As an additional study, fish plasma from caught wild fish in the UK and Germany has been analyzed. Preliminary results from this study show that APIs could be detected in the UK samples (from the lower and upper River Thames catchment), e.g risperidone and flecainide.

Evaluation of WWT

Studies of treatment effectiveness will be conducted with 46 APIs from the priority list. Results from the "chemical map" show that these 46 APIs cover a wide range of chemical properties. Analytical support for these experiments is on-going. Umeå has also assisted Lund in the validation of the pretreatment and handling of samples. Umeå University has also participated in the LTU / DTUs sorption experiments of 75 APIs, these results were presented at the SETAC conference in Seville in 2010 and the manuscript was submitted to Water Research in late 2010.

Analytical Determination

In addition to the wide screening method, several high through-put methods for specific substances that support to other parts of the program have also been developed. These methods are based on-line SPE and reduce sample preparation time and increase capacity significantly. This method development has made it possible to analyze verapamil, levonorgestrel, ethinyl estradiol, perphenazine, ketoprofen and progesterone in a much more efficient manner. This has increased the statistical power of the studies and also made it possible to include a bioconcentration study of progestins.

Umeå University has also been involved in the evaluation of the removal effiency of APIs in different Swedish wetlands together with Stockholm University. Constructed wetlands have a capacity for removing a variety of micro-pollutants, including APIs, and could hence be a resource efficient complement to more advance treatment technologies. Our results show that the selected 92 pharmaceuticals had an average removal rate in the investigated wetlands ranging from 42 to 52%, which is comparable to those of traditional sewage treatment plants. Incoming and outgoing water in the wetlands were also evaluated by ecotoxicity tests. The outcome from these test; EC50s in the range of 7.5 to 46% as the percentage reduction in growth rate compared to the control with macro alga and LOECs in the range of 11.25 to 90% with crustacean, could not be assigned to either pharmaceutical residues or metals, but in general showed that these treatment facilities release water with a relatively low toxic potential, comparable to water that have been treated with advanced tertiary treatments.

We have also participated in a study with the aim to reduce the levels of APIs in hospital effluent by targeting patient urine. This investigation was led by Lund and results are pending.

Method development for improved sludge extraction based on pressurized liquid extraction (PLE) is on-going and this method will be applied to sludge that have been thermally heated and exposed to ozone (in a project led by Lund), sludge from Käppalaverket (in a project together with Käppalaförbundet) and sludge from the national screening programme organized by the Swedish EPA.

A general QA / QC protocol, including sampling, sample handling, stability of standards, etc. have been established. This protocol will be continuously updated and ongoing QA / QC work is also underway to validate the new methods. Umeå University has previously participated in a Swedish collaborative study of the analysis of pharmaceutical compounds organized by the Swedish Water, and researchers at ISPRA will be contacted in order to organize a similar exercise during 2011.

Plans for 2011

During 2011 the ongoing bioconcentration studies will be finalized which will give new and important data for improvement of the fish plasma model. The new data will also be evaluated for refinement of the API ranking list. Several manuscripts will be written and submitted for publication, including new analytical methods. Results will be presented at SETAC 2011.

Analytical determination in various sample matrices will continue as support for all partners within MistraPharma.

Staff

During 2010, the following personnel have been involved to various extent in the activities at Umeå University: Mats Tysklind, Jerker Fick, Richard Lindberg, Roman Grabic, Patrik Andersson, Hanna Söderström, Rolf Andersson, Per Lundholm, Ganna Fedorova, Ghazanfar Khan, and Anna-Lena Lilliehöök.

Publications

Gyllenhammar Irina, Eriksson Hanna, Söderqvist Anneli, Lindberg Richard H., Fick Jerker, Berg Cecilia Clotrimazole exposure modulates aromatase activity in gonads and brain during gonadal differentiation in Xenopus tropicalis frogs Aquatic Toxicology 2009 91, 2:102-9

Söderström H, Lindberg RH, Fick J. 2009. Tools for monitoring emerging polar organic contaminants in water with emphasis on integrative passive sampling. Journal of Chromatography A, 1216, 3:623-30

Larsson DGJ, Fick J. 2009. Transparency throughout the production chain – a prerequisite to reduce pollution from the manufacturing of pharmaceuticals?
Regulatory Pharmacology & Toxicology, 53, 161-163.

Lina Gunnarsson, Erik Kristiansson, Carolin Rutgersson, Joachim Sturve, Jerker Fick, Lars Förlin, and D. G. Joakim Larsson. Pharmaceutical Industry Effluent Diluted 1:500 Affects Global Gene Expression, Cytochrome P4501A Activity and Plasma Phosphate in Fish. Environmental Toxicology and Chemistry. In Press

Fick J., Söderström H., Lindberg R.H., Phan C., Tysklind M., Larsson D.G. J. Contamination of surface, ground and drinking water from pharmaceutical production. Environm. Toxicol. Chem., 2009, 28:2522-2527.

Jerker Fick, Richard H. Lindberg, Mats Tysklind, and D.G.Joakim Larsson. 2010. Predicted critical environmental concentrations of 500 pharmaceuticals. Regulatory Toxicology and Pharmacology. 58, 516-523.

Jerker Fick, Richard H Lindberg, Jari Parkkonen, Björn Arvidsson, Mats Tysklind, D G Joalim Larsson. 2010. Therapeutic Levels of Levonorgestrel Detected in Blood Plasma of Fish: Results from Screening Rainbow Trout Exposed to Treated Sewage Effluent. Environmental Science & Technology. 44, 2661-2666.

Maritha Hörsing, Anna Ledin, Roman Grabic, Jerker Fick, Mats Tysklind, Jes la Cour Jansen . 2011 Determination of sorption coefficients for seventy five pharmaceuticals in sewage sludge. Submitted to Water Research

Cuklev F, Kristiansson E, Asker N, Fick J, Förlin L, Larsson DGJ, 2011. Diclofenac bioconcentrates in fish and affects global hepatic gene expression. Submitted manuscript

Roman Grabic, Jerker Fick, Richard H Lindberg, Ganna Fedorova, Mats Tysklind. 2011 Multi-residue method for trace level determination of 103pharmaceuticals in environmental samples by liquid chromatography coupled to triple quadrupole mass spectrometry. Manuscript in preparation

Patrik L. Andersson, Jerker Fick, Stefan Rännar. 2011. A Multivariate Chemical Similarity Approach to Search for Drugs of Potential Environmental Concern. Submitted manuscript

Breitholtz M, Näslund M, Stråe D, Borg H, Grabic R, Fick J. The removal efficiency of pharmaceuticals in four Swedish free water surface wetlands. Manuscript submitted to Water Research.

Review articals, book chapters and books

Kallenborn, R, J. Fick, R. Lindberg, M. Moe, K. M. Nielsen, M. Tysklind and T. Vasskog. 2008. Pharmaceutical residues in Northern European Environments: Consequences and Perspectives. Book chapter in: Pharmaceuticals in the environment 3rd Ed, (Kuemmerer, K. Ed). Springer Verlag, Germany. pp. 61-74.

Fick J., Grabic R., Lindberg R.H., Larsson D.G.J., and Tysklind M. 2010. Bioconcentration of pharmaceuticals. In: Towards sustainable pharmacy in a healthy society. pp 36-45. ISBN 978-91-978836-0-3.

Conference proceedings with referee board

Mats Tysklind, Richard Lindberg, Roman Grabic, Hanna Söderström, D G Joakim Larsson, Jerker Fick. Pharmaceuticals in the environment – properties, occurrence and environmental fate. 12th EuCheMS International Conference on Chemistry and the Environment (ICCE2009), Stockholm, Juni, 2009.

Jerker Fick, Richard Lindberg, Jari Pakkonen, Mats Tysklind, Joakim Larsson. Bioconcentration of 18 human pharmaceuticals into blood plasma of fish exposed to treated sewage effluents. SETAC Europe 19th Annual Meeting, Göteborg, Juni, 2009.

Jerker Fick, Richard H Lindberg, Roman Grabic, Mats Tysklind. 2010. Bioconcentration of pharmaceuticals in fish plasma. Nordic Environmental Chemistry Conference, Longyearbyen, Svalbard, Mars, 2010.

Roman Grabic, Jerker Fick, Richard H Lindberg, Jenny Rattfelt Nyholm, Frans Schoutsen, Mats Tysklind. Advantages of less usual LC/MS techniques as APPI/APCI (atmospheric pressure photo/chemical ionization) with MS/MS and HRMS- analysis of pharmaceuticals, BFRs, and other new pollutants. Nordic Environmental Chemistry Conference, Longyearbyen, Svalbard, Mars, 2010.

Jerker Fick, Richard H Lindberg, Mats Tysklind, D G Joakim Larsson. Critical environmental concentrations for 500 pharmaceuticals. SETAC Europe 20th Annual Meeting, Seville, May, 2010.

Publications associated projects

Saccà ML, Accinelli C, Fick J, Lindberg R H, Olsen B. 2009. Environmental fate of the antiviral drug Tamiflu in two aquatic ecosystems. Chemosphere, 75, 28-33.

Söderström H, Järhult JD, Olsen B, Lindberg RH, Tanaka H, Fick J. Detection of the antiviral drug Oseltamivir in aquatic environments. 2009 PLoS ONE 4(6): e6064, doi:10.1371/journal.pone, 0006064

Lindberg R., Fick J., Tysklind M. 2010 Screening of antimycotics in Swedish sewage treatment plants – Waters and sludge. Water Research. Water Research, 44, 649-657

Saccà ML, Accinelli C, Fick J, Lindberg RH, Mencarelli M, Olsen B. 2010. Fate and removal of oseltamivir (Tamiflu) in contrasting aquatic environments. Chemosphere 79, 891-897

Accinelli C, Saccà ML, Batisson I, Fick J, Mencarelli M, Grabic R. 2010. Removal of oseltamivir (Tamiflu) and other selected pharmaceuticals from wastewater using a granular bioplastic formulation entrapping propagules of Phanerochaete chrysosporium. Chemosphere 81, 436–443.

Brosche S, Fick J, Larsson DGJ, Backhaus T 2011 Effluent from antibiotic production induce tolerance development in natural freshwater bacterial communities Submitted manuscript

Kristiansson E, Fick J, Janzon A, Grabic R, Rutgersson C, Weidegård B, Söderström H, Larsson DGJ 2011 Antibiotic-contaminated effluent promotes mobile resistance in environmental bacteria PLoS ONE In Press

Holmberg A, Fogel J, Albertsson E, Fick J, Brown JN, Paxéus N, Förlin L, Johnsson J, Larsson DGJ, 2011. Does waterborne citalopram affect the aggressive and sexual behaviour of rainbow trout and guppy? Journal of Hazardous Materials. In Press

Conference proceedings with referee board

Richard Lindberg, Jerker Fick, Mats Tysklind. Screening of antimycotics in sewage treatment plants. SETAC Europe 19th Annual Meeting, Göteborg, Juni, 2009.

Anna-Lena Lilliehöök, Richard H Lindberg, Mats Tysklind, Jerker Fick. SPE method development and LC-MS/MS comparison - on selected antibiotics and antivirals. Nordic Environmental Chemistry Conference, Longyearbyen, Svalbard 1-5 Mars, 2010.

University of Gothenburg

Project leader: Joakim Larsson

Summary of completed research within MistraPharma

Main scientific results

Between January 2010 and now, the Gothenburg team has published 7 original papers on pharmaceuticals in the environment (including aspects on fate, effects, effluent treatment etc), 5 book chapters, 1 review-article and presented a large number of papers at scientific conferences (not listed). In addition we have submitted 4 original articles and have preliminary results for more than a handful of papers in preparation.

Among the highlights is a paper published in Environmental Science and Technology (Samuelsson et al) in collaboration with Stockholm Vatten on metabolic responses in fish exposed to sewage effluents treated with various technologies. Another study (with UmU) describes a method to predict "Critical Environmental Concentrations" of human pharmaceuticals in water, based on read across from mammals to fish. In a third study, again with UmU, we show that levonorgestrel bioconcentrates to therapeutic levels in fish exposed to sewage effluents. The study by Holmberg et al evaluated aggressive and sexual behavior in two fish species exposed to a waterborn citalopram. The paper by Cuklev et al (submitted) applied micro-array technology to demonstrate that diclofenac (an NSAID) affects hepatic gene expression at concentrations found in some surface waters (around 1 μg/L) corresponding to blood plasma levels in fish similar to or below those found in human blood during treatment. This supports the concept of read-across between humans and fish.

In addition, we have generated gene expression data using microarray for studies on fish exposed to 1) another NSAID at different concentrations, 2) up-and down-stream from Skövde sewage treatment plant and 3) fish exposed under rigorously controlled conditions to clean water or to sewage effluent treated by either conventional methodology alone or such effluent treated with a range of add-on technologies including high and low levels of ozone, activated carbon, ozone in combination with a moving bed biofilm reactor or UV-light in combination with hydrogen peroxide. We are confident with the array data produced in these studies, and we have received

informative preliminary data from all three studies. However due to reoccurring major technical problems with our array platform (Geniom- RT analyzer) we eventually felt forced to switch platforms (to Nimblegen) for the rest of our work. The technical repeated problems and major time-consuming troubleshooting efforts unfortunately have lead to delays of analyses. After we finally decided to switch platforms it has taken several people several months optimizing new protocols to reach a stage where we are confident with the output. Fortunately, the quality of the Nimblegen arrays now appear to be excellent. Altogether, this has also meant that part of the costs for analyses has been moved to the 2011 budget.

Meanwhile we have performed controlled exposure experiments for gene expression analyses and bioconcentration for four drugs in Gothenburg. In collaboration with Prof John Sumpter (Brunel Univ. UK) we have taken part in a long-term dose –response reproduction study in fish on two progestins, where we will study gene expression in gonads and brain, while Brunel is focusing on reproductive parameters. We have also performed exposure experiments with two other drugs for studies on bioconcentration and gene expression. One of these, perphenazine, is a neuroactive drugs, so for this drug we have also performed a range of behavioural tests in fish (Adriaenssens et al, in prep). Focus in 2011 will be to complete all of these studies with most of the focus on gene expression analyses and bioconcentration.

Related scientific output, and input for phase II during the past year

In a series of paper from 2007 and onwards, we have demonstrated that direct releases from the manufacturing of drugs, rather than from usage, is the source for the highest levels of drugs in the environment. Particularly our data on releases of broad spectrum antibiotics in India have raised serious concerns about pollution from drug manufacturing as a driver for antibiotic resistance, a challenge that lack national borders. We have also established that the production of drugs intended for the Swedish market, contributes to massive pollution in India.

We recently developed a novel method, based on open shotgun massively parallel pyrosequencing of community DNA, to analyze the presence of thousands of different resistance genes in parallel in a sample, such a as a complex environmental bacterial community (Kristiansson et al, 2011). Applying this exploratory method, we showed that antibiotic-contaminated river sediments in India contained very high levels of both resistance genes for several classes of antibiotics as well as different gene transfer elements, including plasmids, transposons and integrons (Kristiansson et al, 2011).

Given the ability of resistance genes to transfer horizontally between species, and the flow of bacteria between different milieus, our result stress the risk that antibiotic pollution from drug manufacturing could contribute to the development of multi-resistant pathogens, one of the three largest threats to public health globally, according to the WHO.

Accordingly, the Gothenburg team has during the past two years started to switch focus from gene expression in fish as their core-competence area, to metagenomics, DNA-sequencing and environmental antibiotic resistance. This has been possible partly through new recruitments and collaborations, and there are now several papers in the pipeline along this line of research. Consequently, MistraPharma will take advantage of this acquired competence and put more focus on the assessments of antibiotic resistance in the environment in phase II. As a step in this direction, MistraPharma has during 2010-11 funded pilot projects to evaluate and perform 1) DNA extraction and amplification from bacterial communities, 2) array-based enrichment strategies of resistance genes prior to sequencing, 3) pilot sequencing run with Illumina technology to increase sensitivity and 4) PCR analyses of selected resistance genes. Projects 1, 3 and 4 show promising results, although we have abandoned the array-enrichment protocol suggested, but we are in the other hand considering other enrichment strategies.

Staff

In addition to the staff previously active within MistraPharma in Gothenburg, there has been some recruitments, including Lina Gunnarsson, Bethanie Carney-Almroth, Marija Svijovic, Bart Adriaenssens and Carl-Fredrik Flach, all postdoctoral fellows in the areas of fish physiology, fish behavior, molecular biology and/or bioinformatics (analyses of array-data). Input has also been required from other staff in the group that are usually more involved in other projects, including Anders Janzon, particularly for implementing protocols for the new array platform.

Postdoctoral fellows Erik Kristiansson and Marija Svijovic both received Assistant Professorships 2011 at Chalmers in high competition. Also, Bart Adriaessens received a research position in Australia. We congratulate them to these achievements and wish them luck in their new positions. We foresee a continued collaboration for MistraPharma phase II, particularly with Kristiansson and his new group at Chalmers.

Publications

Original-articles

Cuklev F, Kristiansson K, Fick J, Asker N, Förlin L, Larsson DGJ. 2011. Waterborne diclofenac bioconcentrates in fish and affects global hepatic gene expression at blood plasma concentrations similar to human therapeutic levels. Environmental Toxicology and Chemistry. Accepted with revisions.

Lennquist A, Asker N, Kristiansson E, Brenthel A, Björnsson B-T, Kling P, Larsson DGJ, Förlin L. 2011. Physiology and gene expression in rainbow trout (Onchorhyncus mykiss) after long- term exposure to the new antifoulant medetomidine. Accepted with minor revisions in Comparative Biochemistry and Physiology

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Rutgersson C, Gunnarsson L, Kristiansson E, Larsson DGJ. 2010. Short-term exposure to effluent from bulk drug industries does not indicate acute toxic effects in rats. Submitted.

Kristiansson E, Fick J, Janzon A, Grabic R, Rutgersson C, Weijdegård B, Söderström H, Larsson DGJ 2011. Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. PLoS ONE. PLoS ONE 6(2): e17038.

Holmberg A, Fogel J, Albertsson E, Fick J, Brown JN, Paxéus N, Förlin L, Johnsson J, Larsson DGJ. 2011. Does waterborne citalopram affect the aggressive and sexual behaviour of rainbow trout and guppy? Journal of Hazardous Materials. 187:596-599.

Samuelsson LM, Björlenius B, Förlin L and Larsson DGJ. 2011. Reproducible 1H NMR-based metabolomic responses in fish exposed to different sewage effluents in two separate studies. Environmental Science and Technology. 45:1703–1710.

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Fick J, Lindberg RH, Tysklind M, Larsson DGJ. 2010. Predicted Critical Environmental Concentrations for 500 Pharmaceuticals. Regulatory Toxicology and Pharmacology. 58: 516-523.

Fick J, Lindberg RH, Parkkonen J, Arvidsson B, Larsson DGJ. 2010. Therapeutic levels of levonorgestrel detected in blood plasma of fish: Results from screening rainbow trout exposed to treated sewage effluents. Environmental Science and Technology. 44:2661-2666.

Albertsson E, Larsson DGJ and Förlin L. 2010. Induction of hepatic carbonyl reductase/20β-hydroxysteroid dehydrogenase mRNA in rainbow trout downstream from sewage treatment works – possible roles of aryl hydrocarbon receptor agonists and oxidative stress. Aquatic Toxicology. 97:243-249.

Gunnarsson L, Adolfsson-Erici M, Björlenius B, Rutgersson C, Förlin L, Larsson DGJ. 2009. Comparison of six different sewage treatment technologies - reduction of estrogenic substances and effects on gene expression in exposed male fish. Science of the Total Environment 407:5235-5242.

Gunnarsson L, Kristiansson E, Rutgersson C, Sturve J, Fick J, Förlin L, Larsson DGJ. 2009. Pharmaceutical industry effluent diluted 1:500 affects global gene expression, CYP1A activity and plasma phosphate in fish. Environmental Toxicology and Chemistry 28:2639-2647.

Fick J, Söderström H, Lindberg RH, Chau DNP, Tysklind M, Larsson DGJ. 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. Environmental Toxicology and Chemistry. 28:2522-2527.

Larsson DGJ and Fick J. 2009. Transpararency throughout the production chain – a way to reduce pollution from the manufacturing of pharmaceuticals. Regulatory Toxicology and Pharmacology. 53:161-163.

Carlsson G, Örn S and Larsson DGJ. 2009. Effluent from bulk drug production is toxic to aquatic vertebrates. Environmental Toxicology and Chemistry 28:2656-2662.

Gunnarsson L, Jauhiainen A, Kristiansson E, Nerman O, Larsson DGJ. 2008. Evolutionary conservation of human drug targets in organisms used for environmental risk assessments. Environmental Science and Technology 42(15):5807-5813. (Received the "AstraZeneca Award for the best publication in risk assessment, modeling and theoretical studies" in 2008)

Reviews

Larsson DGJ. 2011. Release of active pharmaceutical ingredients from manufacturing – what do we know and what are the risks? Manuscript submitted to Läkartidningen (in Swedish).

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Samuelsson L.M and Larsson D.G.J. 2008. Contributions from metabolomics to fish research. Molecular Biosystems. 4, 974–979.

Book chapters

Janzon A, Kristiansson K, Larsson DGJ. 2011. Environmental microbial communities living under very high antibiotic selection pressure. Invited book chapter in: Antimicrobial Resistance in the Environment. Eds. Montforts MH, Keen P. Wiley & Blackwell. In press.

Larsson DGJ and Lööf L. 2011. Läkemedel i miljön. Invited book chapter (In Swedish) to appear in "Läkemedelsboken", edited and produced by the Swedish Medical Products Agency.

Gunnarsson L, Kristiansson E and Larsson DGJ. 2011. Environmental Comparative Pharmacology: Theory and application. Invited book chapter to appear in: Human Pharmaceuticals in the Environment: Current and Future Perspectives. Eds: B Brooks, D Huggett. Springer Verlag.

Cuklev F, Gunnarsson L, Larsson DGJ. 2010. Genomics as a guide to assess environmental risks of pharmaceuticals. In: Towards sustainable pharmacy in a healthy society. Pp 58-71. ISBN 978-91-978836-0-3.

Fick J, Grabic R, Lindberg RH, Larsson DGJ, Tysklind M. 2010. Bioconcentration of pharmaceuticals. In: Towards sustainable pharmacy in a healthy society. Pp 36-45. ISBN 978-91-978836-0-3.

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novel testing approaches. Pp 148-165. In: A healthy future – pharmaceuticals in a sustainable society. Eds: Bengtsson B-E et al. Apoteket AB. ISBN: 2184-01.

Larsson DGJ. 2008. Drug production facilities – an overlooked discharge source for pharmaceuticals to the environment. In Kümmerer K, ed, Pharmaceuticals in the Environment. Sources, Fate, Effects, and Risks. Springer, New York, NY, USA, pp 37-42.

Reports

Swedish Board for Health and Welfare 2011. Government commision to the Swedish Board for Health and Welfare for a new national strategy to manage antibiotic resistance. Available at http://www.socialstyrelsen.se/publikationer2011/2011-3-14 (In Swedish). Larsson contributed to the parts relating to the external environment.

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Breitholtz M, Larsson DGJ. 2009. Summary of ecotoxicological evaluations of studies at Sjöstadsverket and Henriksdals sewage treatment plant 2007 and 2008. Report to Stockholm Vatten AB. Available at : http://www.stockholmvatten.se/sv/Aktuellt/Projekt/Lakemedel-i-avloppsvatten/

Swedish Environmental Protection Agency 2008. Government comission to the Swedish Environmental Protection Agency on the ability of sewage treatment plants to treat pharmaceutical residues and other toxic chemicals. 512-386-06 RmRapport 5794. (Larsson was consulted to write part of the report). Available at www.naturvardsverket.se

PhD-theses

Gunnarsson L. 2009. On the use of genomics to assess environmental risks of pharmaceuticals. Doctoral dissertation. The Sahlgrenska Academy at the University of Gothenburg. ISBN 978-91-628-774.

Alexandra Jauhiainen. 2010. Statistics in Gene Expression, Metabolomics, and Comparative Genomics in Evolution. PhD thesis. Chalmers University of Technology, Sweden.

Licenciate thesis

Jauhiainen A. Microarray analysis of mRNA decay assays and prediction of drug target conservation. 2008. Licenciate thesis. Department of Mathematical Sciences. University of Gothnburg and Chalmers University of Technology. ISSN 1652-9715.

Half-time controls (similar to Licenciate degree)

Cuklev F. Genomics as a Guide for Environmental Risk Assessments of Pharmaceuticals. April 15, 2010. Department of Neuroscience and Physiology, Sahlgrenska academy at the University of Gothenburg.

Rutgersson C. Environmental effects of pollution from drug manufacturing. 17 Feb, 2011. Department of Neuroscience and Physiology, Sahlgrenska academy at the University of Gothenburg.

Teaching - undergraduates and practioners

Larsson and Gunnarsson have introduced and taught "pharmaceuticals in the environment" during 2010 and 2011 on about 8 undergraduate educational programs in Gothenburg, including for example the Medical Doctors Programme, two Pharmacy-programmes, the Odonotology-programme, Nursing programmes and more. Gunnarsson has recently implemented a pedagogic format of "interrupted lecturing" on this topic, which involves the students more than regular lectures, and this format was highly appreciated by the students.
On request from the Swedish Medical Agency, Larsson has together with Lars Lööf, medical doctor in Västerås, authored a book chapter on "Pharmaceuticals in the Environment" in "Läkemedelsboken 2011" (The Pharmacy-book 2011). This book is used as a pharmaceutical reference book for practicing doctors in Sweden as well as course literature on numerous educational programmes at Swedish Universities.

Larsson and Gunnarsson also teach "pharmaceuticals in the environment" to practicing doctors, nurses, and high-school teachers both at the Sahlgrenska Academy, Chalmers University of Technology and the Nordic School for Public Health (NHV).

Uppsala University

Project leader: Ingvar Brandt

Summary of completed research within MistraPharma

Six original papers have been published since January 2010. Another three manuscripts are in pipeline to be submitted for publication. Three licentiate theses are being prepared and expected to be presented in May/June 2011. Six master theses are to be presented.

Among the most important results is the recent finding that the contraceptive gestagen Levonorgestrel (LNG) is a potent and efficient developmental toxicant in female Xenopus tropicalis frogs (Kvarnryd et al. 2011a, Aquatic Toxicol). In adult female frogs exposed to LNG during the tadpole stage, the ovaries displayed an increased proportion of eggs arrested in meiotic prophase. At two doses examined, the lowest one approaching environmentally measured LNG concentrations, a complete lack of developed oviducts was observed. Hence, the differentiation of the embryonic precursor to the oviduct, the Müllerian duct, in frogs is a sensitive target for gestogens as well as previously demonstrated for estrogens (Pettersson et al, 2006; Gyllenhammar et al, 2009). The LNG-exposed females ovulated directly into the abdominal cavity, and no offspring was generated when exposed females were paired with nonexposed males. Consequently, the females were sterile, while no developmental effect of LNG on the male reproductive system could be observed in this study. Another notable finding was that no developmental effect was visible at metamorphosis, directly after the LNG exposure had been discontinued. The implication of this finding is that a full life-cycle test seems necessary to disclose the severe consequences of developmental exposure to this type of compounds. Studies are in progress to define the lowest LNG concentration affecting female sex organ development, and relate this concentration to those previously demonstrated in STP effluent water in Sweden. Reproductive effects of LNG in frogs exposed as adults have also been evaluated (Kvarnryd et al. 2011b, manuscript). It was of particular interest to note that following adult exposure, unlike developmental exposure, the spermatogenesis of the males was impacted. LNG may thus be a reproductive toxicant in adult male frogs while it is a developmental toxicant in female frogs.

The usefulness of the frog test system to detect neuroendocrine disruption has been confirmed following developmental exposure to fluoxetin, a selective serotonin reuptake inhibitor and antidepressant human pharmaceutical. Following developmental exposure, no effects on sex differentiation, estrogen synthesis or the thyroid system were observed. Interestingly, however, there were altered regional concentrations of certain transmitter substances/metabolites in the brains of metamorphosed frogs, implying that human neuroactive pharmaceuticals may also affect neurodevelopmental variables in Xenopus tropicalis frogs (Berg et al 2011).

The pharmacokinetics of LNG is currently being investigated in fish and frogs. As recently demonstrated by autoradiography, there is a rapid absorption of LNG from ambient water into the fish. There is also a rapid and pronounced excretion of LNG into the bile and intestinal contents, implying a rapid metabolic turnover of LNG in fish. A targeting of LNG/LNG metabolites to certain endocrine organs may shed light on the mechanism of action of LNG in fish.

A variety of biomarkers have been developed or established in order to characterize various effects of pharmaceuticals and their interaction with other pollutants in fish. An interesting observation is that LNG seems to be a potent androgen in fish. Studying another classical biomarker, vitellogenin, the expression of the vitellogenin gene seems to be epigenetically modified following exposure of zebrafish to the potent environmental estrogen, ethinylestradiol (EE2) (Strömquist et al. 2010, Aquatic Toxicol). Exposure of adult zebrafish to EE2 decreased the methylation level of CpG sites in the promoter region of the vitellogenin gene in the liver in both females and males.

Working with cytochrome P450 biomarkers, we observed that certain drugs (particularly antimycotic azoles) were efficient inhibitors of CYP1A-catalysed enzyme activity, i.e. EROD activity (Beijer et al.2010, Aquatic Toxicol). In the further work, we have cloned the whole suite of CYP1 forms in rainbow trout, stickleback and Xenopus tropicalis, and examined their inducibility by standard inducers and STP effluent water in Sweden (Jönsson et al. 2011, Aquatic Toxicol a;b; Gao et al.2011, Comp Biochem Physiol). From this work we have strong indications that particularly the CYP 1C genes are responsive to pharmaceuticals. They show strong induction at the Uppsala STP but weak response at the Skövde STP. In collaboration with the Gothenburg group, we currently examine effluent water collected at Patancheru (Beijer et al. 2011, manuscript). Results show that highly diluted Patancheru water can both inhibit CYP-catalysed enzyme activity and strongly induce

CYP genes. A method to measure effects of pharmaceuticals on gene expression in gill filaments in vitro has been developed (Behrens, Master thesis 2011). Recent findings in the model indicate that azoles have a strong influence on CYP 1C expression.

Plans for 2011

Three Licenthiate theses will be finalized during 2011. The work on frogs will continue to define a no-effect concentration for developmental toxicity of LNG in this species. Studies are also ongoing to establish reproductive toxic effects of LNG in frogs exposed as adults. A study to define the androgenic potential of LNG in adult stickleback will be carried out. Likewise, the first developmental study in zebrafish will commence. The biomarker tool-box genes recently developed will be applied to characterize the impact of individual APIs and mixtures in experimentally exposed fish. The usefulness of this biomarker approach will be evaluated in fish caged at selected hotspots outside STPs in Sweden and in the Haihe river, Tianjin, China.

Staff

The following personell are currently involved, some of them supported by faculty funding and external sources. Project leader: Ingvar Brandt, Deputy project leader: Björn Brunström, Researchers: Cecilia Berg, Maria Jönsson PhD students: Kristina Beijer, Kai Gao, Moa Kvarnryd, Johan Svensson Laboratory engineer: Margareta Mattsson.

Publications

Original articles and manuscripts to be submitted

Beijer K, Gao K, Jönsson M, Larsson DGJ, Brunström B, Brandt I (2011) Biomarker responses in three-spined stickleback (Gasterosteus aculeatus) exposed to diluted effluent from a treatment plant in Patancheru, India, heavily contaminated with pharmaceuticals. Manuscript in preparation.

Kvarnryd M, Eriksson A, Norder A, Fick J, Berg C (2011b). Impacted gametogenesis in Xenopus tropicalis frogs after exposure to low concentrations of levonorgestrel. Manuscript in preparation.

Berg C., Backström, T, Winberg, S, Lindberg, R, Brandt, I. Developmental Exposure to Fluoxetine or Estrogen Modulates the Serotonin and Dopamine Systems in the brain of Xenopus tropicalis. Manuscript in preparation.

Kvarnryd, M., Grabic, R., Brandt, I., Berg, C. (2011a) Early life progestin exposure causes arrested oocyte development, oviductal agenesis and sterility in adult Xenopus tropicalis frogs, Aquatic Toxicol., 103, 18-24.

Jönsson M, Berg C, Goldstone J, Stegeman J. (2011b) New CYP1 genes in the frog Xenopus (Silurana) tropicalis: Induction patterns and effects of AHR agonists during development. Toxicology and Applied Pharmacology, 250:170-83.

Jönsson ME, Gao K, Olsson JA, Goldstone JV, Brandt I (2011a) Induction patterns of new CYP1 genes in environmentally exposed rainbow trout. Aquatic Toxicol., 98, 311-321..

Gao K, Brandt I, Goldstone JA, Jönsson ME (2011) Cytochrome P450 1A, 1B, and 1C mRNA induction patterns in three-spined stickleback exposed to a transient and a persistent inducer. Comp. Biochem. Physiol. C. www.ncbi.nlm.nih.gov/pubmed/21354474.

Beijer K, Abrahamson A, Brunström B, Brandt I (2010) A method to measure inhibition of cytochrome P450 1A activity in fish gills – application on pharmaceuticals and other aquatic contaminants. Aquatic Toxicol., 96, 145-150.

Strömqvist M, Tooke N, Brunström B (2010) DNA methylation levels in the 5′ flanking region of the vitellogenin I gene in liver and brain of adult zebrafish (Danio rerio)–Sex and tissue differences and effects of 17α-ethinylestradiol exposure. Aquatic Toxicol. 98, 275-281.

Berg C, Gyllenhammar I, Kvarnryd M (2009) Xenopus tropicalis as a Test System for Developmental and Reproductive Toxicity. J Tox Environ Health, 72:219-225.

Gyllenhammar I, Eriksson H, Söderqvist A, Lindberg R, Fick J, Berg C (2009a). Clotrimazole exposure modulates aromatase activity in gonads and brain during gonadal differentiation in Xenopus tropicalis frogs. Aquat Toxicol 91:102-109.

Gyllenhammar I, Holm L, Eklund R, Berg C (2009b). Reproductive Toxicity in Xenopus tropicalis after Developmental Exposure to Environmental Concentrations of Ethynylestradiol. Aquat toxicol, 91:171-178.

Ph D thesis

Gyllenhammar I. 2008. Endocrine Disruption in Amphibians. Ph.D. thesis. Department of Environmental Toxicology, Uppsala University.

Master of Science theses

Norder, A. 2011. Effects on oogenesis in Xenopus tropicalis frogs after exposure to low concentrations of levonorgestrel. Master programme in environmental toxicology, Uppsala university. In preparation

Eriksson, A. 2011. Impacted Spermatogenesis in Xenopus tropicalis frogs after exposure to low concentrations of levonorgestrel. Master programme in environmental toxicology, Uppsala university. In preparation

Svensson, J. 2011. Effects of the synthetic progestin levonorgestrel on zebrafish (Danio rerio) reproduction. Report no. 140. Master programme in environmental toxicology, Uppsala university.

Behrens, D. 2011. Cytochrome P450 1 induction and inhibition in rainbow trout (Oncorhynchus mykiss) gills -A new in vitro method applied on pharmaceuticals. Master programme in environmental toxicology, Uppsala university. In preparation

Eriksson H. 2010. Effekter av etinylöstradiol på aromatasaktiviteten i gonader och hjärna hos Xenopus tropicalis under könsdifferentieringen. Master programme in environmental toxicology, Uppsala university.

Andersson, M. 2010. Effekter av det antiöstrogena läkemedlet tamoxifen på könsdifferentieringen hos Xenopus tropicalis. Åbo Akademi, Finland/Dept Environmental Toxicology, Uppsala University.

Söderqvist, A. 2008. Effekter av fluoxetin och östrogen på metamorfosen och könsdifferentieringen hos Xenopus tropicalis. MSc program in Aquatic and Environmental Engineering, Uppsala University.

Kvarnryd, M. 2008. Effekter av clotrimazol på könsdifferentiering och aromatasaktivitet I gonader och hjärna hos Xenopus tropicalis. Master programme in environmental toxicology, Uppsala University.

Book chapters, theses, proceedings, reports

Kvarnryd M. 2011. Developmental reproductive toxicity of levonorgestrel in the frog Xenopus tropicalis. Licentiate thesis. In preparation.

Berg C. 2011. Environmental Pollutants – A threat towards reproduction in amphibians?" Proceedings, CRU Report 25, Uppsala.

Berg C. 2011. An Amphibian Test System for Developmental and Reproductive Toxicity. In Methods in Molecular Biology. Eds. Harris C, and Hansen JM. Submitted for publication.

Berg C, Kvarnryd M, Grabic R, Brandt I. 2011. Early life progestin exposure causes sterility in adult female Xenopus tropicalis frogs. Proceeding SETAC, 2011.

Kristina Beijer. 2010. Human drugs as environmental contaminants -Pharmaceuticals affecting the cytochrome P450 system in fish. Licentiate thesis, in preparation.

Kai Gao. 2010. Expression patterns of different CYP1 isoforms in fish and application in biomonitoring in aquatic ecosystem. Licentiate thesis, in preparation.

Berg C. 2010. The Frog Test System. In "Towards Sustainable Pharmaceuticals in a Healthy Society". Eds. Rudén, Liljelund & Hagerman. Year book by MistraPharma, Erlanders Sverige AB, Stockholm Sweden.

Brandt I., Brunström B. 2010. Laboratory vs Field Studies to Assess Environmental Hazards and Risks Posed by Pharmaceuticals for Human Use. In "Towards Sustainable Pharmaceuticals in a Healthy Society". Eds. Rudén, Liljelund & Hagerman. Year book by MistraPharma, Erlanders Sverige AB, Stockholm Sweden.

Berg C. 2009. Developmental reproductive toxicity of estrogenic environmental pollutants in amphibians and birds. Proceeding SETAC 2009.

Presentations at international conferences (oral or poster)

Berg C, Kvarnryd M, Grabic R, Brandt I. Early life progestin exposure causes sterility in adult female Xenopus tropicalis frogs. SETAC (International Society of environmental toxicology and chemistry), Milan, Italy, 2011. Oral.

Beijer K, Gao K. Jönsson ME. Brunström, Larsson DGJ, Brandt I. Highly diluted effluent from drug manufacturing induces cytochrome P450 1 mRNA expression in three-spined stickleback (Gasterosteus aculeatus). Primo 16: Pollutant Responses in Marine Organisms 15-18 may 2011, Long Beach, California USA

Kvarnryd, M. A. Eriksson, A. Norder, C. Berg. 2011. Impacted Spermatogenesis in Xenopus tropicalis frogs after exposure to low concentrations of levonorgestrel. 16th PRIMO conference, Long Beach, USA, 2011.

Jönsson, ME, Berg C, Goldstone J, Stegeman J. 2010. Induction of CYP1 genes and effects of AHR agonists in Xenopus tropicalis tadpoles. Society of Toxicology, USA, 2010.

Jönsson, ME, Berg C, Goldstone J, Stegeman J. 2010. Induction of CYP1 genes and effects of AHR agonists in Xenopus tropicalis tadpoles. Society of Toxicology, USA, 2010.

Berg C. Xenopus tropicalis – a test system for environmental pollutants. Center for Chemical Pesticides Symposium: Risk assessment and effect studies of pesticides. SLU, 2009.

Berg C., Backström, T, Winberg, S, Lindberg, R, Brandt, I. Developmental Exposure to Fluoxetine or Estrogen Modulates the Serotonin and Dopamine Systems in Xenopus tropicalis. SETAC, 2009. Oral spotlight.

Beijer K, Abrahamson A, Brunström B, Brandt I: Antifungal drugs and omeprazole inhibit CYP1 activity in three-spined stickleback gills and larvae. SETAC 2009, Göteborg. Poster spotlight.

Hjalmarsson M, Tooke N, Brunström B: Effects of ethinylestradiol on DNA methylation in a 5-primeflanking region of the vitellogenin I gene in adult zebrafish (Danio rerio). IUTOX 2010, Barcelona. Abstractet är publicerat I Toxicology Letters 196S(2010)S117.

Kvarnryd, M., Gyllenhammar, I., Fick, J., Lindberg R, Berg, C. Endpoints for Sexual Disruption in Xenopus tropicalis: Effects of ethynylestradiol and clotrimazole on aromatase activity and gonadal differentiation. SETAC, 2009.

Berg, C. Xenopus tropicalis as a test system for developmental reproductive toxicity. Environmental Toxicology Symposium, Norway, 2008. Oral.

Other presentations and lectures on pharmaceuticals in the environment

Berg, C. Seminar at the Center for Reproductive Biology, Uppsala, February 2011. "Environmental Pollutants – A threat towards reproduction in amphibians?"

Berg, C, Brandt I. Uppsala University, MSc programme in Clinical Pharmaceutics, "Effects of pharmaceuticals on aquatic wildlife", 2008, 2009, 2010.

Berg, C. Landstingens nätverk för läkemedel och miljö, Uppsala, November 2009. "Sex-reversal in frogs – an effect of pharmaceuticals in the environment".

Svensson J, Brunström B, Brandt I, Berg C, and others, Various courses in toxicology, ecotoxicology and biology at Uppsala University

Stockholm University

Project leader: Magnus Breitholtz

Summary of completed research within MistraPharma

Standard testing of APIs

The contribution from Stockholm University to the programme has mainly focused on delivering standard ecotoxicological test data using micro algae (OECD 201), Daphnia magna (OECD, 202 and 211) and fish embryos (OECD 2006[1]) to the synthesis project. The ambition from start of the programme was to generate standard data for 15-30 Active Pharmaceutical Ingredients (APIs). At the end of 2010, the following number of APIs had been tested in different standard tests: micro algae (19), Daphnia acute (17), Daphnia chronic (10 st) and fish embryo (17). In addition, the project has during 2010 complemented these tests with a novel feeding inhibition test (8) and a gene expression test (3) with Daphnia. Overall, and as expected, the standard tests have been relatively insensitive to the APIs prioritized within the programme.

To complement our standard life cycle data, in particular generated with the chronic tests with D. magna, we have applied all data to an individual based population model (IBM). The use of such models facilitates generation of population level data, which is preferred in environmental risk assessment. They are also useful for evaluating test endpoint sensitivity and are thus a valuable tool for optimizing test designs and identifying specifically sensitive endpoints. However, since most APIs tested so far within the programme have had relatively weak potency in the chronic Daphnia test, it is too early to judge whether the population level data generated by the IBM adds significant relevance and/or sensitivity for testing APIs in general.

The bulk of the data from the standard tests mentioned above (including population level and feeding inhibition data) will be incorporated in a joint programme publication to be published in the end of 2011 (Asp et al., manuscript in preparation).

[1] OECD 2006. Fish Embryo Toxicity (FET) Test. OECD guideline for the testing of chemicals, draft proposal for a new guideline.

Development of a new IBM model

In close collaboration with German colleagues, our work within the programme has also lead to the development of a new IBM (Preuss et al., submitted manuscript), which is based on the experimental design in a draft OECD Test Guideline for harpacticoid copepods (OECD, 2009[2]). Originating from the Mistra research programme NewS, a Swedish initiative was taken under the work plan of the OECD Test Guideline Programme to develop and validate a test guideline on development and reproduction of marine/brackish copepods (Kusk and Wollenberger, 2007[3]). Although this standardization work is currently focused on Amphiascus tenuiremis, our work within MistraPharma has shown that also the brackish water harpacticoid Nitocra spinipes can be used to generate consistent data by following the proposed test method (Lundström et al., 2010; Preuss et al., submitted manuscript; Lundström et al., manuscript). By using the new copepod IBM, we have shown that the experimental design in the draft OECD harpacticoid copepod TG can be simplified in terms of reduced number of replicates and longer endpoint inspection intervals, without losing statistical power (Preuss et al., submitted manuscript). Of general importance for the field, a major contribution from this work is that the use of IBMs could drastically reduce the time for developing new standard tests, which may take as long as 10-15 years (Breitholtz et al., 2010). Since our work within the MistraPharma synthesis project has shown that lack of relevant standard tests hampers environmental risk assessment of pharmaceuticals (Ågerstrand et al., submitted manuscript), these findings are of major importance for the programme as a whole.

Cellular and molecular responses of APIs

A main objective has also been to identify cellular and molecular effects based on the fact that some drug targets also in other species than vertebrates (i.e. fish) have been conserved during evolution. For this purpose we have exposed D. magna to three selected priority APIs in experiments where expression of selected genes have been analyzed using qPCR. So far, we have found significant responses in transcription level of genes related to reproduction (vitellogenin) and moulting (cuticle protein) (Furuhagen et al.,

[2] OECD. 2009. OECD Draft Guidelines for Testing of Chemicals. Proposal for a New Guideline. Harpacticoid Copepod Development and Reproduction Test. OECD.
[3] Kusk KO, Wollenberger L. 2007. Towards an internationally harmonized test method for reproductive and developmental effects of endocrine disrupters in marine copepods. Ecotoxicology 16:183-195.

manuscript). Taken out of the causal context, gene expression changes are in general not easily interpretable and may thus be difficult to use for risk assessment. Hence, we are currently relating these responses to the standard acute and chronic data generated for D. magna, as well as to responses on individual growth (RNA/DNA ratios, body length), heart rate and oxidative status. The working hypothesis is that alterations in transcription are manifested both earlier and at a lower exposure levels as compared to effects observed at organism level (e.g. reproduction).

Evaluation of wastewater treatment technologies

A further ambition within the programme has been to evaluate advanced wastewater treatment technologies for improved removal of APIs. In close collaboration with Lund/DTU and Umeå we are evaluating the usefulness of ozone but also chlorine dioxide. Our preliminary results indicate that both treatment technologies may generate transformation products that could be equally or more potent than the parent APIs when applied to synthetic mixtures of APIs. Considering the strong oxidation, structural changes/ transformations are highly likely and as complement to the standard tests we propose to apply markers of oxidative stress.

In collaboration with Umeå and four municipal sewage treatment plants (STPs), in 2010 we also evaluated the removal efficiency of four constructed wetlands as polishing steps after conventional sewage treatment (Breitholtz et al., submitted manuscript). APIs were analyzed in both incoming and outgoing wastewater from the wetlands. In addition, the same ecotoxicological test battery as was used in the study by Lundström et al. (2010a) was used to characterize the wastewaters. Chemical analysis of 94 selected APIs showed that, under cold Scandinavian winter conditions, the average removal rates from the four investigated constructed wetlands are comparable to those of traditional STPs. The findings from the ecotoxicological testing were less useful than the chemical analysis for the overall purpose of the study in the sense that other substances than pharmaceuticals were responsible for the observed toxicities (e.g. ammonium). Still, the ecotoxicological tests showed that in general the four investigated treatment facilities release wastewater with a relatively low toxic potential. In fact, the effect levels obtained in the present study are comparable to effect levels from tested effluent wastewaters from a much larger treatment facility in Stockholm (i.e. Henriksdal), equipped with more advanced treatment techniques (i.e. ozone, active carbon, UV/H_2O_2) currently suggested as promising treatment technologies for removal of pharmaceuticals (Lundström et al., 2010a). From the submitted study it was concluded that constructed wetlands may provide a cost effec-

tive and relevant wastewater treatment option, especially where treatment of wastewater is lacking today.

Activites within the MistraPharma synthesis project

Apart from the important findings on the use of IBMs presented above we have participated in the MistraPharma synthesis project to: 1) investigate if non-standard ecotoxicity data can be evaluated systematically in risk assessments of pharmaceuticals (Ågerstrand, Breitholtz and Rudén, submitted manuscript) and 2) present a new set of reliability and relevance evaluation criteria for environmental risk assessment of pharmaceuticals (Ågerstrand et al., submitted manuscript).

Plans for 2011

In 2011, we will focus on writing manuscripts and finalizing the standard testing of the prioritized APIs. Two reproduction tests with Daphnia will be performed and the remaining APIs will be tested with the Daphnia feeding inhibition test. We will also continue with the evaluation of the promising wastewater treatment technologies in collaboration with Lund/Umeå. Finally, we will attend the SETAC conference in Milan, where we will present three MistraPharma-related posters.

Staff

In 2010, the following personnel have been involved in the project:

- Magnus Breitholtz, project leader, 10%, supervision, writing manuscripts.
- Elin Lundström, PhD student, 100%, experimental work, writing manuscripts, part time parental leave.
- Karin Ek, technician, 90%, responsible for Daphnia tests.
- Margareta Linde, technician, 20%, responsible for micro algae and fish embryo tests.
- Sara Furuhagen, technician, 100%, responsible for evaluation of wastewater treatment technologies, worked with Daphnia tests.

Publications

Published manuscripts

Lundström E, Brinkmann M, Hollert H, Persson J-O, Breitholtz M. (2010a) Comparison of six sewage effluents treated with different treatment technologies – population level responses in the harpacticoid copepod Nitocra spinipes. Aquatic Toxicology 96(4), 298-307.

Lundström E, Adolfsson-Erici M, Alsberg T, Eklund, Lavén M, Breitholtz M. (2010b). Characterization of additional sewage treatment technologies: Ecotoxicological effects and levels of selected pharmaceuticals, hormones and endocrine disruptors. Ecotoxicology and Environmental Safety 73, 1612-1619.

M, Breitholtz M, Rudén C. Comparison of four different methods for reliability evaluation of ecotoxicity data - A case study of non-standard test data used in environmental risk assessments of pharmaceutical substances. Accepted for publication in Environmental Sciences Europe a.

Submitted manuscripts

Breitholtz M, Näslund M, Stråe D, Borg H, Grabic R, Fick J. The removal efficiency of pharmaceuticals in four Swedish free water surface wetlands. Manuscript submitted to Water Research.

Preuss TG, Brinkman M, Lundström E, Bengtsson B-E, Breitholtz M. An individual –based modeling approach for evaluation of endpoint sensitivity in harpacticoid life cycle tests. Manuscript submitted to Environmental Toxicology and Chemistry.

Ågerstrand M, Breitholtz M, Rudén C. Comparison of four different methods for reliability evaluation of ecotoxicity data - A case study of non-standard test data used in environmental risk assessments of pharmaceutical substances. Manuscript submitted to Environmental Sciences Europe a.

Ågerstrand M, Küster A, Bachmann J, Breitholtz M, E. ert I, Rechenberg B, Rudén C. Reporting and evaluation criteria as means towards a transparent use of ecotoxicity data for environmental risk assessment of pharmaceuticals. Manuscript submitted to Environmental Pollution b.

Manuscripts in preparation

Roos V, Ågerstrand M, Rudén C. Sensitivity of standard vs. non-standard tests for ecotoxicological testing of APIs.

Breitholtz M, Rudén C, Lindström K, Fick J. Single and mixture toxicity of four pharmaceuticals (Cyp-inhibitors) in the harpacticoid copepod Nitocra spinipes.

Lundström, E., Breitholtz, M, Preuss, T. Comparison of matrix and individual based population modeling on harpacticoid copepods – A case study on complex effluents and lindane.

Furuhagen S, Gorokhova E, Lundström E, Preuss TG, Fick J, Ek K, Fuchs A, Breitholtz M. Coupling gene expression to individual and population level responses in Daphnia magna exposed to three pharmaceuticals with different modes of action.

Book chapters

Rudén C, Brandt I, Breitholtz M, Fick J, la Cour Jansen J, Larsson J, Tysklind M (2009) Feminiserade fiskar och förgiftade fåglar – läkemedelsrester i miljön innebär nya ekotoxikologiska utmaningar. I ”En frisk framtid – Läkemedel i ett hållbart samhälle”. (Bengtsson, Gunnarsson, Hagerman, Liljelund och Wennmalm eds.), Apoteket AB, MistraPhamra och Stockholms Läns Landsting, ISBN: 2182-01,Stockholm, Sweden, 198p. (Is also available as an English version: ”A healthy future – Pharmaceuticals in a sustainable society”).

Rudén C, Ågerstrand M, Göransson M, och Breitholtz M. (2010). “Standard and non-standard tests for risk assessment purposes” MistraPharma årsbok 2009.

Breitholtz, M., Lundström E., Dahl U., Forbes, V. (2010). Improving the value of standard toxicity test data in REACH. In: Regulating Chemical Risks. European and Global Challenges (Eds.: Eriksson, Johan; Gilek, Michael; Rudén, Christina). 1st Edition, Springer, ISBN: 978-90-481-9427-8.

Conference contributions

Lindström K, Lundström E, Fick J, Breitholtz M (2009) Effects of mixtures of pharmaceuticals with known mode of action using standard endpoints in crustaceans and algae. Poster presentation. SETAC Europe 19th Annual Meeting 31 May – 4 June 2009, Gothenburg, Sweden.

Lundström E, Gorokhova E, Breitholtz M (2009) Population responses and growth-related endpoints in Daphnia pulex exposed to pharmaceuticals. Poster presentation. SETAC Europe 19th Annual Meeting 31 May – 4 June 2009, Gothenburg, Sweden.

Lundström E, Brinkmann M, Dahl U, Ek K, Björlenius B, Wahlberg C, Breitholtz M (2009) Copepod population modeling as a tool for evaluating novel sewage water treatment techniques. Poster presentation. SETAC Europe 19th Annual Meeting 31 May – 4 June 2009, Gothenburg, Sweden.

Breitholtz, M (2010) Are standard tools needed for reliable regulatory hazard and risk assessment? Invited speaker at JRC/EES workshop: Aquatic Ecotoxicology – can we improve its influence on policies and risk management? European Environment Agency, Copenhagen 6-7th of May 2010.

Breitholtz M, Lundström E, Bengtsson B-E, Preuss TG (2010). Modelling as a tool to get more meaningful data from biotests - an experimentalistic point of view. Poster presentation. SETAC Europe 20th annual meeting, 23-27th May, Seville, Spain.

Ågerstrand M, Breitholtz M, Rudén C. (2010) Evaluation of the reliability of ecotoxicity data - a comparison of four different methods. Poster presentation. SETAC Europe 20th annual meeting, 23-27th May, Seville, Spain.

Küster A, Ågerstrand M, Bachmann M, Breitholtz M, Ebert I, Rudén C, Rechenberg B (2010) Proposal for a new reliability and relevance evaluation scheme for ecotoxicity data. Poster presentation. SETAC Europe 20th annual meeting, 23-27th May, Seville, Spain.

Lundström E, Gorokhova E, Preuss TG, Breitholtz M (2010) Comparison of traditional and novel endpoints for environmental risk assessment of active pharmaceutical ingredients in Daphnia magna. Poster presentation. SETAC Europe 20th annual meeting, 23-27th May, Seville, Spain.

Lundström E, Breitholtz M, Brinkmann M, Preuss T.G. (2010) A comparison of matrix and individual based modeling using harpacticoid copepods, SETAC North America 2010, 7-11th of November, Portland, Oregon.

Lund University/DTU

Project leaders: Jes la Cour Jansen, Anna Ledin

Summary of completed research within MistraPharma

Initially the test methods for the most promising treatment techniques were developed. All the central treatment techniques included in the program have been tested with respect to a limited number of carefully chosen pharmaceuticals.

In parallel with development of methodologies for testing the treatment techniques, have great efforts been made together with Umeå University (UmU) in order to develop a procedure that would make it possible to conduct experiments with 120 prioritized pharmaceuticals in relevant concentration levels. Analytical results have so far been obtained only for a subset of the 120 pharmaceuticals in two sets of experiments. These are i) sorption of pharmaceuticals to sludge and powdered activated carbon (PAC), and ii) oxidative treatment using chlorine dioxide.

Analytical results are available for 75 of the 120 pharmaceuticals in the experiments investigating sorption of pharmaceuticals to sludge and powdered activated carbon (PAC). The obtained results from the sorption experiment using sludge have made it possible to calculate the sorbed fraction and thereby describe the partitioning of the pharmaceuticals between sludge and water. As a consequence of the sorption to sludge the order of priority for substances with high tendency for bioaccumulation will change, since they are not expected to reach the aquatic environment. The study was presented at the annual SETAC meeting 23-27 of May in Sevilla, Spain.

Sorption to PAC shows great effectiveness to largely reduce all pharmaceuticals with small doses of PAC (10 mg/L). By using PAC in the secondary treatment step in a laboratory scale reactor it was shown that PAC was enmeshed in the sludge and the technology can therefore be implemented without reconstruction or expansion of the existing treatment plants. Calculations related to the costs of running the treatment options indicate that treatment using PAC may be comparable with ozone treatment for reduction of most of the pharmaceuticals. Outcome of this work will be two publications of which the first is under review in Water Research, and the other will be submitted soon. By these the sorption experiments are finalised.

The first experiments with chlorine dioxide treatment of the 120 API mix have been performed. Analytical results are available for 53 out of the 120 substances. The results have been compiled and submitted to a conference where it was accepted for an oral presentation. The experiment describes the relationship between the treatment doses of chlorine dioxide and the reduction of the analyzed pharmaceuticals. The dose of chlorine dioxide needed for reduction of the pharmaceutical show great variation between different pharmaceuticals. This means that some pharmaceuticals will not be significantly reduced even by a chlorine dioxide dose of 20 mg/L, which is considered to be a technically and economically unrealistic high treatment dose. However, with a treatment dose in technically and economically relevant levels, 2 – 4 mg/L, highly relevant pharmaceutical such as EE2 and diclofenac can be efficiently reduced.

A methodology for testing the ozone treatment technique has been developed and the first experiments with the mix containing many pharmaceuticals are will be performed during the spring 2011.

The test method for biological treatment techniques was developed during the autumn 2009, and the first experiments with the 120 mix was performed beginning 2010. However, the results showed significant variability why the results could not easily be interpreted. Several experiments have been performed in order to find a procedure that is suitable for fewer substances (50) still in relevant concentration levels. Efforts to develop a useful sample handling procedure are ongoing, and recently the practices have been revised. This work will continue during 2011. Consequently, only limited results are available concerning degradation of the program's prioritized pharmaceuticals in system based on activated sludge and biofilm carriers.

We have performed experiments with a small set of pharmaceuticals in parallel with the 120 mix. These analyses have been performed at DTU. The experiments have been very successful and the results demonstrate new knowledge – significant differences in the pattern of biological degradation between activated sludge and biofilm carriers. The results clearly show that biofilm carriers have an enhanced capability compared to activated sludge for reducing these pharmaceuticals by biodegradation. The selection of pharmaceuticals includes molecules that are considered to be persistent in wastewater treatment. To assess the relevance of these results to a broader range of API, experiments with a larger number of substances (20-50) is planned for spring 2011. If this method proves equally efficient for other (types of) pharmaceuticals, then we expect that it will be possible to come up with suggestions for upgrading of biological wastewater treatment plants

which will make these systems robust and with an enhanced reduction of pharmaceuticals.

LU/DTU has participated in a number of strategic initiatives within the program:

Funds granted to LU/DTU and University of Stockholm (SU) for: Will transformation products formed during chemical treatment cause hazardous effects in the environment? The content of the project is i) chlorine dioxide and ozone treatment of tap water spiked with pharmaceuticals, ii) chlorine dioxide and ozone treatment of wastewater and iii) chlorine dioxide and ozone treatment of wastewater spiked with pharmaceuticals. Chemical treatment was performed with several doses followed by standard test using Daphnia magna and growth inhibition of algae.

LU/DTU is responsible for the chemical treatments and SU for the ecotoxicological tests. The experiment with treatment of spiked tap water and wastewater including the ecotoxicological tests were performed during the autumn 2010. Preliminary results indicate that ozone reduces the toxicity caused by the pharmaceuticals completely, while the results from the chlorine dioxide treatments imply that there might be problems concerning formation of toxic compounds. In order to confirm the implied results and to be able to publish the results, the experiment needs to be repeated.

In addition LU/DTU have participated in three smaller projects partly financed by the MistraPharma strategic funds:

UmU, Käppalaförbundet and LU/DTU: ”Undersökning av kemicondprocessens resp. avvattningens påverkan på slammets innehåll av läkemedelssubstanser vid Käppala avloppsreningsverk.” The granted funds should cover analyses of pharmaceuticals in wastewater, reject water and sludge before and after kemicond treatment. LU/DTUs part was to contribute to the interpretation of the results. So far we have not been asked to contribute.

LU/DTU and UmU: ”Effekten av ozonbehandling och termisk behandling av rötslam för metanutbyte och nedbrytning av läkemedel bundet till slam”. This is a project financed by LU however, MistraPharma contributed with funds for analyses of pharmaceuticals at UmU. The project was performed during the summer 2010 and analyses are currently being finalized.

LU/DTU and UmU: “Hospitalsspildevand – Bedst tilgængelige teknik og forrensnings-metoder” (see below) was granted funds for extended ana-

lytical contribution at UmU in order to get detailed knowledge regarding treatment of urine and biological treated wastewater from hospitals using activated carbon and three different oxidation techniques. The project has been performed and the analytical results were delivered shortly before Christmas. The Report will be put together during 2011.

Other activities of relevance for MistraPharma

LU/DTU and UmU have been collaborating with some Danish partners in the Danish project "Hospitalsspildevand – Bedst tilgængelige teknik og forrensnings-metoder". In the project a few of the treatment techniques and analytical methods developed within MistraPharma has been tested. The treatment techniques have been applied to biological treated wastewater from a hospital with high concentration of pharmaceuticals and to urine from the hospitals special units. Most of the project has been performed during the year and the final report was in principal approved by the projects board shortly before Christmas. The report will be published in the series of publications from the Danish Environmental Protection Agency in 2011.

Plans for 2011

The experimental protocols for all central methods regarding separation of pharmaceuticals from wastewater have been developed and experiments have been performed using a small assortment of pharmaceuticals. The efforts together with Umeå to develop a sampling handling procedure that would make it possible to conduct experiments with the prioritized pharmaceuticals in relevant concentration levels is ongoing and the progress of these efforts will affect the planning and achievements during 2011. We hope to be able to finalize the ongoing activities during 2011 and plan to make the following publications:

- Antonio, M., Moradas, G., et al., Removal of XX API with ozone, experiments to be done
- Falås, P., et al., Examination of fate of pharmaceuticals in Swedish WWTP, manuscript under preparation.
- Falås, P., Baillon-Dhumez, A., Andersen, H. R., Ledin, A., la Cour Jansen, J. Removal of seven acidic pharmaceuticals by biofilm carriers and activated sludge, manuscript under preparation for Water Research.
- Hörsing, M., et al., Will transformation products formed during chemical treatment cause hazardous effects in the environment, experiments to be done.

LU/DTU are planning a seminar regarding treatment techniques. The seminar will take place in Lund during the autumn 2011.

The final report and remaining experimental protocols will be prepared during the autumn.

Report and protocol regarding reduction of pharmaceuticals using physical treatments by applying membrane techniques is planned to be performed during the autumn.

Staff

The major part of the research regarding treatment techniques are performed by the two PhD-students and one post doc. Per Falås dedicate his research to biological methods and integration of processes, Gerly Moradas dedicate her research to reduction of pharmaceuticals in the wastewater treatment plants by means of chemical oxidation techniques. Maritha Hörsing has moved from DTU to LU in order to finish the research regarding sorption of pharmaceuticals to sludge and to PAC, furthermore she works with evaluation of the risk for formation of transformation and by products during chemical treatment in the purpose to reduce pharmaceuticals. Three senior researchers Professor Jes la Cour Jansen, LU, professor Anna Ledin adj. Professor at LU and assoc. Professor Henrik Andersen DTU participates to the research by supervising and by their own projects. Laboratory assistance is provided by Ylva Persson, Gertrud Persson and Christina Maj Hagberg. If necessary special competence is associated to the program, as is the case for membrane technology. A great number of bachelor and master degree projects have been generated during the year.

Publications

Falås, P., Andersen, H.R., Ledin, A., la Cour Jansen, J. Impact of solid retention time and nitrification capacity on the ability of activated sludge to remove pharmaceuticals, submitted manuscript Environmental Technology.

Hörsing, M., Ledin, A., Grabic, R., Fick, J., Tysklind, M., la Cour Jansen, J., Andersen, H.R. Determination of sorption coefficients for seventy five pharmaceuticals in sewage sludge, submitted manuscript Water Research.

Falås, P., Baillon Dhumez, A., Andersen, H. R., Ledin, A., la Cour Jansen, J. Removal of seven acidic pharmaceuticals by biofilm carriers and activated sludge, manuscript in preparation.

Hörsing, M. et al., Sorption isotherms obtained for pharmaceuticals in presence of powder activated carbon (PAC) during wastewater treatment, manuscript in preparation.

Moradas, G., Ledin, A., la Cour Jansen, J., Andersen, H. R., Removal of active pharmaceutical ingredients in biologically treated wastewater by chemical oxidants, manuscript in preparation

Moradas, G., Ledin, A., la Cour Jansen, J., Andersen, H. R., Oxidation of mixed active pharmaceutical ingredients in biologically treated wastewater, manuscript in preparation.

Reports

Nielsen et al., Hospitalsspildevand – Bedst tilgængelige teknik og forrensnings-metoder to be published in the report series of the Danish Environmental Protection Agency.

Falås, P., et al., Examination of fate of pharmaceuticals in Swedish WWTP, manuscript in preparation.

Falås, P., la Cour Jansen, J. Swedish Wastewater Treatment Plants and Their Ability to Remove Pharmaceuticals, In Towards Sustainable Pharmaceuticals in a Healthy Society, MistraPharma Research. Ed. Christina Rudén, Karin Liljelund and Helene Hagerman. ISBN 978-91-978836-0-3 (2010).

Falås, P och la Cour Jansen, J. Svenska reningsverk och deras processuppbyggnad i relation till reduktion av läkemedel. Rapportmanuskript (2008).

Book chapters

Falås, P., la Cour Jansen, J. Swedish Wastewater Treatment Plants and Their Ability to Remove Pharmaceuticals, In Towards Sustainable Pharmaceuticals in a Healthy Society, MistraPharma Research. Ed. Christina Rudén, Karin Liljelund and Helene Hagerman. ISBN 978-91-978836-0-3 (2010).

Chapter 8. Rudén, C., I. Brandt, M. Breitholtz, J. Fick, J. la Cour Jansen, K. Liljelund, D.G.J.Larsson, M Tysklind. Feminized Fish and Vulnerable Vultures – Pharmaceuticals as Environmental Pollutants Require Novel Testing Approaches Human pharmaceuticals in surface waters – novel types of environmental pollutants require novel testing approaches. In Rudén, C., I. Brandt, M. Breitholtz, J. Fick, J. la Cour Jansen, K. Liljelund, D.G.J. Larsson, M Tysklind. A Healthy Future Pharmaceuticals in a Sustainable Society. ISBN:2184-01 (2009).

Chapter 9. J. la Cour Jansen and A. Ledin. Sustainable Wastewater Treatment. In Rudén, C.,I. Brandt, M. Breitholtz, J. Fick, J. la Cour Jansen, K. Liljelund, D.G.J. Larsson, M Tysklind. A Healthy Future Pharmaceuticals in a Sustainable Society. ISBN:2184-01 (2009).

Masters Thesis projects and other reports

Baillon-Dhumez A. (2010). "Evaluation of a Biofilm Process for the Removal of Pharmaceuticals in Wastewater". Thesis project

Kerlin, N. (2010). "NSAID- oxidation with PAA". Bachelor thesis.

Hastrup, C. (2010). "Citalopram- oxidation with Fenton". Special project for elite student.

Tatari, K. (2010). "Fate of E2 and EE2 in aerobic ammonium oxidizing biofilters". Thesis project.

Kong X. (2010). "Fate of several lipid regulator and non-steroidal anti-inflammatory drug (NSAID) substances in aerobic ammonium oxidizing biofilters". Thesis project.

Lindberg S. (2008). ”Nedbrytning av läkemedelsrester i avloppsreningsverk”. Thesis project.
Säfström C. (2008). “Reduction of active pharmaceutical ingredients and oestrogens in wastewater using powdered activated carbon”. Thesis project.
Söderberg T. (2008). “Powdered activated carbon used as adsorbent of active pharmaceutical ingredients and estrogens in the biological treatment of wastewater”. Thesis project.

Conferences and seminars

Falås, P., Baillon-Dhumez, A., Andersen, H. R., Ledin, A., la Cour Jansen, J. Removal of seven acidic pharmaceuticals by biofi lm carriers and activated sludge, Micropol & Ecohazard 2011, the 7th IWA specialist conference on assessment and control of micropollutants/hazardous substances in water, Sydney 11-13 Juli, 2011. Oral presentation.

Furuhagen, S., Hörsing, M., Ledin, A., Andersen, H.R., Gorokhova, E., Breitholtz, M. Identification of potential toxicity caused by O3 and ClO2 treatment of pharmaceuticals in wastewater, 21st SETAC Europe annual meeting, 15-19 May in Milano, Italy. Poster.

Hörsing, M., Furuhagen, S., Ledin, A., Breitholtz, M., Andersen, H.R. Identifi cation of ecotoxicity caused by O3 and ClO2 treatment of wastewater, 20th Ozone World Congress and 6th Ultraviolet World Congress, 23 - 27 May in Paris, France. Poster presentation.

Moradas, G., Ledin, A., la Cour Jansen, J., Andersen, H. R., Oxidation of mixed active pharmaceutical ingredients in biologically treated wastewater, 20th Ozone World Congress and 6th Ultraviolet World Congress, 23 - 27 May in Paris, France. Oral presentation.

Hörsing, M., Andersen, H.R., la Cour Jansen, J., Ledin, A., Sorption to sludge- Fate of pharmaceuticals in wastewater treatment plants. 20th SETAC Europe annual meeting, Seville, Spain.

Rudén, C., I. Brandt, M. Breitholtz, J. la Cour Jansen, J. Larsson, K. Liljelund and M.Tysklind. MistraPharma - Identification and Reduction of Environmental Risks Caused by the Use of Human Pharmaceuticals. Short oral presentation and poster at IWA World Water Congress, Wien 2008.

KTH

Project leader: Christina Rudén

Summary of completed research within MistraPharma

The work of the KTH-team during 2010 has been organized into four subprojects:
(1) Evaluation of the Swedish voluntary system for environmental information about API on fass.se,
(2) evaluation of the usefulness of standard vs. non-standard ecotoxicological data for environmental risk assessment of API,
(3) evaluating the sensitivity of standard vs. non-standard data for identifying API of ecotoxicological concern, and
(4) evaluation of different priority setting criteria for selecting API for eco toxicological testing.

In addition to this, KTH is also responsible for maintaining and updating of the WikiPharma database.

The first two subprojects are included in Marlene Ågerstrand´s licentiate thesis that was defended in October 2010. The title of the thesis is: Improving the transparency and predictability of environmental risk assessments of pharmaceuticals. The thesis covers two main areas: first an evaluation of the Swedish voluntary system for environmental information about API on fass.se, and second the usefulness of standard vs. non-standard ecotoxicological data for environmental risk assessment of API. The project is co-financed by Formas.

The first paper in the thesis reports from an empirical investigation of the motivations, intentions and expectations underlying the development and implementation of a voluntary industry owned environmental classification system for pharmaceuticals. The results show that the purpose of the classification system is to provide information; no other risk reduction measures are aimed for.

The second paper reports from an evaluation of the accuracy and the consistency of the environmental risk assessments conducted within the classification system. The results show that the guideline recommendations were not followed in several cases and consequently alternative risk ratios could be

determined for six of the 36 pharmaceutical substances selected for evaluation in this study. When additional data from the open scientific literature was included the risk ratio was altered for more than one-third of the risk assessments. Seven of the 36 substances were assessed and classified by more than one risk assessor. In two of the seven cases, different producers classified the same substance into different classification categories.

The third paper addresses the question whether non-standard ecotoxicity data could be used systematically in environmental risk assessments of pharmaceuticals. Four different evaluation methods were used to evaluate nine non-standard studies. The evaluation result from the different methods varied at surprisingly high rate and the evaluation of the non-standard data concluded that the reliability of the data was generally low.

Sensitivity of standard vs. non-standard tests for ecotoxicological testing of API

This project is performed in collaboration with Magnus Breitholtz (Stockholm University).

In this project we use the data compiled in the WikiPharma database as well as data generated within MistraPharma to compare the sensitivity of standard and non-standard tests for identifying API of ecotoxicological concern. The method for such systematic comparison has been developed and analyses are currently ongoing. Preliminary results show considerable differences in the sensitivity between standard and non-standard tests for a significant number of the API. However, for some API the standard tests seem more sensitive, but for others the non-standard tests are the more sensitive ones. This first overview will be supplemented with information about the API´s mode-of-action and an analysis of whether the observed results depend on the specificity of the effect and endpoint.

These additional analyses will be performed during 2011 and a manuscript finalized.

The MistraPharma prioritization process in perspective

This project is performed in collaboration with Jerker Fick (Umeå University), Lina Gunnarsson and Joakim Larsson (Göteborg University).

The purpose of this project is to put the MistraPharma prioritization process in perspective by comparing it to other attempts to prioritize API for ecotoxi-

cological testing. In this project a database has been compiled. The database contains information for little less than 600 APIs. The selection of substances was based on the following criteria: The API is registered in Sweden (2010), the API is covered by the EMA requirements for an environmental risk assessment, and the API is designed to target eukaryote cells. The compiled data includes for instance sales statistics, pharmacological data, ecotoxicological data. Data from WikiPharma and from fass.se have been included.

The analyses of this database is currently ongoing. We use the database to rank API according to different criteria proposed in the scientific literature, including the fish plasma model as used in the MistraPharma prioritization process. The outcomes of these different methods/criteria will be compared, analyzed and discussed. Preliminary conclusions indicate that the relative potency of the API, the molecule´s degradability, and its potential for bioconcentration are important aspects that need to be taken into account at first tier to identify API that should be further tested for ecotoxicological effects.

A manuscript from this project will be finalized during 2011.

Vendela Roos participated in a workshop in Leipzig on the use of therapeutic data for environmental risk assessment of pharmaceuticals in August 2010.

WikiPharma - a database for ecotoxicity data for pharmaceuticals

The WikiPharma database was created in 2009 and updated in January 2010 and March 2011. The database currently contains ecotoxicity data from the open scientific literature for 149 pharmaceutical substances from 209 references. This compilation confirms that data are still missing for a majority of the pharmaceutical substances.
E-mail correspondence with researchers and regulators from several countries in Europe and North America indicates that the database is considered a useful and important tool in the work with environmental risk assessments of pharmaceuticals.

Plans for 2011

During 2011 we will finalize the ongoing analyses reported above and the manuscripts that are currently in preparation.

Staff

Christina Rudén, Marlene Ågerstrand and Vendela Roos.

Publications

Peer reviewed articles

Ågerstrand, M and C Rudén (2010) "Accuracy and consistency of the Swedish Environmental Classification and Information System for pharmaceuticals" Science of the Total Environment (STOTEN) 408:2327-2339.

Molander L, Ågerstrand M, and Rudén C. (2009). "WikiPharma - a freely available, easily accessible, interactive and comprehensive database for environmental effect data for pharmaceuticals" Regulatory Toxicology and Pharmacology 55(3):367-371.

Ågerstrand M, Wester M, and Rudén C. (2009). "The Swedish Environmental Classification and Information System for Pharmaceuticals — An empirical investigation of the motivations, intentions and expectations underlying its development and implementation" Environment International, 35:778–786.

Ågerstrand M, Breitholtz M, and Rudén C. (2010). Comparison of four different methods for reliability evaluation of ecotoxicity data-A case study of non-standard test data used in environmental risk assessments of pharmaceutical substances. Accepted for publication in Environmental Sciences.

M, Breitholtz M, Rudén C. Comparison of four different methods for reliability evaluation of ecotoxicity data - A case study of non-standard test data used in environmental risk assessments of pharmaceutical substances. Accepted for publication in Environmental Sciences Europe a.

Manuscripts

Ågerstrand M, Küster A; Bachmann J, Breitholtz M, Ebert I, Rechenberg B, Rudén C. Reporting and evaluation criteria as means towards a transparent use of ecotoxicity data for environmental risk assessment of pharmaceuticals. Submitted.

Roos V, Ågerstrand M, Rudén C. Sensitivity of standard vs. non-standard tests for ecotoxicological testing of APIs. In preparation.

Roos V, Fick J, Gunnarsson L, Larsson DGJ, Rudén C. Comparing prioritisation schemes for environmental risk assessment of human pharmaceuticals (working title). In preparation.

Licentiate thesis

Ågerstrand M. 2010. Improving the transparency and predictability of environmental risk assessments of pharmaceuticals. Licentiate Thesis in Risk and Safety, Royal Institute of Technology, Stockholm. ISBN: 978-91-7415-737-6

Book chapters

Rudén C, Ågerstrand M, Göransson, M, and Breitholtz M. (2010). "Standard and non-standard tests for risk assessment purposes" MistraPharma yearly report 2009.

Molander L, Ågerstrand M, and Rudén C. (2010) "WikiPharma – A database with environmental effect data for pharmaceuticals" MistraPharma yearly report 2009.

Ågerstrand M and Rudén C. (2010). "The Swedish Environmental Classification and Information System for pharmaceuticals as a mean for risk management. An evaluation of the system's achievements so far" MistraPharma yearly report 2009.

Rudén C, Brandt I, Breitholtz M, Larsson J, La Cour Jansen J, Tysklind M. "Feminized fish and vulnerable vultures – Pharmaceuticals as environmental pollutants require novel testing approahes" in A Healthy Future. Pharmaceuticals in a Sustainable Society. ISBN 2184-01, financed by MistraPharma, Apoteket AB and Stockholm County Council, March 2009. Also available in Swedish: "En frisk framtid – Läkemedel i ett hållbart samhälle" ISBN 2182-01.

Masters thesis projects and other reports

Pilenvik M (2009). "Classification and labelling as a tool for reducing chemical risks - A qualitative comparison of four systems". Candidate Thesis.

Molander L. (2009). "The Swedish environmental risk classification system for pharmaceuticals – An analysis of current classifications and criteria for data selection". Masters Thesis project in collaboration with Lund university.

Östensson A. (2008) "Environmental Risk Assessment of Ibuprofen. Comparison of the risk assessment procedures according to EMEA, REACH, and Fass.se". Project thesis.

Conference contributions

Roos V. Comparing prioritisation schemes for environmental risk assessment of human pharmaceuticals. Accepted for oral presentation at SETAC, May 2011.

Ågerstrand M. "Standard and non-standard tests for environmental risk assessment of human pharmaceuticals". Accepted for oral presentation at SETAC, May 2011.

Küster A, Ågerstrand M, Bachmann J, Breitholtz M, Ebert I, Rudén C, Rechenberg B. (2010). Proposal for a new reliability and relevance evaluation scheme for ecotoxicity data. Poster presented at SETAC Europe 2010 in Seville, Spain.

Ågerstrand M, Breitholtz M, Rudén C. (2010). Comparison of four different methods for reliability evaluation of ecotoxicity data - A case study of non-standard test data used in environmental risk assessments of pharmaceutical substances. Poster presented at SETAC Europe 2010 in Seville, Spain.

Pilenvik M, Ågerstrand M, Rudén C. (2010). Classification and information as tools for reducing chemical risks - A qualitative comparison of four systems. Poster presented at SETAC Europe 2010 in Seville, Spain.

Rudén C. (2009). "Towards a theory of tiered testing as approach within ITS" Oral presentation at the 7th WC on alternative test methods in Rome 1-4 September 2009.

Rudén C. (2009). "Ten proposals to improve testing and risk assessment" Poster/Abstract and oral presentation at Eurotox September 2009.

Ågerstrand M, and Rudén C. (2009). "Reliability of voluntary environmental risk classifications of pharmaceuticals". Poster/Abstract SETAC Europe May 2009.

Ågerstrand M, Wester M, and Rudén C. (2008). The Swedish environmental classification and information system for pharmaceuticals - an empirical investigation of the motivations, intentions and expectations underlying its development and implementation. Poster/Abstract SETAC Europe 2008.

Ågerstrand M, Wester M, and Rudén C (2008) The Swedish environmental classification and information system for pharmaceuticals - an empirical investigation of the motivations, intentions and expectations underlying its development and implementation. Oral presentation at SETAC/GDCh - Neue Problemstoffe in der Umwelt: Erfassung, Wirkungen, Lösungen. Frankfurt am Main, Germany, September 2008.

Ågerstrand M, Wester M, Rudén C (2008) The Swedish environmental classification and information system for pharmaceuticals - an empirical investigation of the motivations, intentions and expectations underlying its development and implementation. Oral presentation at KNAPPE (Knowledge and Need Assessment on Pharmaceutical Products in Environmental waters). Nimes, France, February 2008.

Rudén C, Brandt I, Breitholtz M, la Cour Jansen J, Hällbom L, Larsson J, and Tysklind M. (2008). "A new research programme: MistraPharma – Identification and Reduction of Environmental Risks Caused by the Use of Human Pharmaceuticals". Poster "Forum för Miljöforskning" Stockholm.

Rudén C, Brandt I, Breitholtz M, la Cour Jansen J, Hällbom L, Larsson J, and Tysklind M. (2007). "A new research programme: MistraPharma – Identification and Reduction of Environmental Risks Caused by the Use of Human Pharmaceuticals". Poster ERAPharm, York 2007.

Publications in associated projects

Hansson SO, Molander L, Rudén C "The substitution Principle" Accepted for publication in Regulatory Toxicology and Pharmacology.

Nordberg A, Rudén C, and Hansson SO. (2008). "Towards more effi cient testing strategies - analyzing the efficiency of toxicity data requirements in relation to the criteria for classifi cation and labelling". Regulatory Toxicology and Pharmacology 50:412-419.

Rudén C. and Hansson SO. (2008). "Comment on: Janer, G., Hakkert, B.C. Slob, W., Vermeire, T., Piersma, A.H. 2007. "A retrospective analysis of the two-generation study: What is the added value of the second generation?" (Reproductive Toxicology 24 (2007) 97-102) Reproductive Toxicology 25:397-405.

Communication project

Project leader: Karin Liljelund

The communication project is responsible for the internal and external communications with a focus on networking and communication with the stakeholders.

During the year, many activities have been carried out to ensure that knowledge of MistraPharma has been communicated with all important stakeholders. The main activities are as follows:

Website

The website is a vital link in contact with our various stakeholders and researchers. Ongoing work, articles, seminars, etc. are continuously posted on the website, along with links to other works and activities in the field of pharmaceuticals and the environment. During the autumn, the website has been developed and made more user friendly.

International and national networks

During the year, both the national and the international network have been expanded. A contact with the networks is done primarily through our newsletter and website.

The reference group

MistraPharma have a dedicated and knowledgeable reference group of 18 different representatives from our most important stakeholders. The reference group is a vital link to ensure that the outcomes of the program will benefit our stakeholders. Due to changes in the pharmacy market the previous representative from Apoteket AB was, during the spring, replaced by a representative from the newly established branch Swedish Pharmacy Association.

In addition to regular contact with the majority of our representatives in the group, following activities was organised during the year:

Meetings

March 24
The meeting was attended by 16 of the reference group's 18 members and MistraPharma program director and the communication project manager. The purpose of this meeting was to find means of cooperation to best utilize the expertise available within the reference group. As a basis for this meeting were telephone interviews with all the reference group members and researchers from a number of predetermined questions. The meeting was very constructive and fruitful. All members of the reference group were to present how they see that they can contribute to the program. That statement was sent to the reference group and all project managers. The outcome of the meeting was that we found ways of how we can best come to take advantage of the expertise available within the reference group.

September 30
The purpose of this meeting was to obtain feedback on the Phase 2 application and also to discuss the format and content of the yearbook for 2010 with a stakeholder focus. The valuable suggestions on the direction for Phase 2, which came up during the meeting were compiled and used as input for the project leader meeting in October where the focus of Phase 2 would be discussed.

Telephone meetings

To further increase the opportunities for interaction between the reference group and MistraPharma telephone meetings began during the year, between regular meetings, based on requests from the reference group.
The purpose of these meetings is for the program director to inform of ongoing work within the program and the various members of the reference group to inform about ongoing or upcoming activities within respective organization. During the year, telephone meetings were held 3 June and 4 November.

Annual books

2008

MistraPharma annual report for 2008 consists of the book "A Healthy Future - Pharmaceuticals in a Sustainable Society" along with a brief summary of the various sub-projects and financial accounting. The book presents a summary of the current knowledge of the problems that surround pharam-

ceuticals and sustainable development. The book has been printed in Swedish (6000 copies) and in English (3000 copies) and have been distributed to our national and international networks in spring 2009.

2009

MistraPharma annual report for 2009 consists of the book "Towards Sustainable Pharmaceuticals in a Healthy Society MistraPharma research" along with a brief summary of the various sub-projects and financial accounting. The book presents some of the research to date generated within MistraPharma. The book has been printed in English in 1000 and copies have been distributed to our national and international networks in spring 2010.

2010

MistraPharma annual report for 2010 consists of the book "MistraPharmas Stakeholders' views on the journey towards Sustainable Pharmaceuticals in a Healthy Society " along with a summary og the various projects and a financial report. The aim of this book is to provide good examples of what is being done or planned in the field of pharmaceuticals and environment of our major stakeholders and to demonstrate cooperation and benefits of MistraPharma. Both a English and Swedish version will be produced and printed.

Seminars

Pro et contra at KTH

In February 2010, a "pro et contra" seminar was held at KTH -"Droger på drift"- Utsläpp av läkemedel från produktionsanläggningar skadar miljön i utvecklingsländer, vad kan vi göra åt saken? The seminar was well attended with about 40 participants. MistraPharma were well represented at the seminar by project managers and representatives from the reference group. Christina Rudén started with "Drugs in the environment, what's the problem? Joakim Larsson informed of their studies in India and how Indian pharmaceutical pollute water. Charlotte Unger gave the Medical Products Agency's' view of how we can ensure clean production of Swedish pharmaceuticals? Camilla Nykvist informed of what AstraZeneca is doing to ensure a clean production of pharmaceuticals. Theres Olsen concluded by saying how the council works as a requirement opener. The seminar was concluded with a panel discussion. The magazine "Ny Teknik" and Mistra newsletter has had reports from the seminar.

The Toxicological Council

During the spring a seminar with focus on pharmaceuticals and the environmental was arranged by the Toxicological Council. 70 people attended and presentations was held by people from MistraPharma Board, Reference Group and Project leaders.

″From a tablet to the toilet”

The seminar was very well attended by over 110 participants including representatives from MistraPharma all important stakeholders. The seminar was conducted as a journey from the manufacture of pharmaceutical products to release through the toilet. The focus of the day was to show what different organizations today can concretely do to reduce the load of pharmaceuticals in our water ways. With the help of a “tour guide”, environmental journalist Charlotte Permell, a discussion were held with the following representatives from various organizations throughout the chain from the tablet to the toilet; LIF - the research-based pharmaceutical industry in Sweden, the Medical Products Agency, TLV- the Dental and Pharmaceutical Benefits Agency, Blekinge County Council, the Pharmaceutical Committee in Västmanland, the Ministry of Health and Social Affairs, the Swedish Pharmacy Association, Svensk dagligvaruhandel, MistraPharma and the Stockholm County Council. The day alternated with very fruitful discussions with seminar participants.

To ensure that the participants at the seminar “From tablet to the toilet” received maximum benefit from what was discussed during the day, documentation was sent out to all participants. The dossier contained a brief summary of the various presentations and group discussions and the presentations (power-point pictures) that were presented during the day.

Information

Brochure

In the spring, we produced, at the request of the reference group, an information leaflet on MistraPharma in Swedish - “MistraPharma-Risks of pharmaceuticals in the aquatic environment”. The booklet presents the overall objectives of the programme and presents some quotes from what some of our key stakeholders like MistraPharma. MistraPharma project leaders and other information about MistraPharma is also presented. The brochure has been distributed to all important stakeholders.

Newsletter

During the year two newsletters have been published in English, according to the program plan, The first in May and the second in December. The newsletters have been distributed via email to all the contacts in our national and international network. A number of copies have also been printed to be distributed at seminars, conferences and more.

Other communication activities

Remittance

MistraPharma has answered a remiss from the National Board of Health and Welfare concerning the use of pharmaceuticals on a deregulated pharmacy market.

Meetings

Karin Liljelund and Christina Rudén had a meeting with Marie Uhrwing the secretary general at the Environmental Objectives Committee

Christina Rudén has had meetings with the following organizations during the year:
- The Swedish Environmental Protection Agency concerning the strategy for the East See.
- The Swedisk Management Council
- Presentation of the program for the Nordic Water Authorities (WFD), the County Council in Sörmland and the Pharnacy and Therapeutidic Committies.

MistraPharma researchers 2010

January
Chistina Rudén, Jerker Fick Filip Cuklev and Joakim Larsson submitted input to the HELCOM meeting (3-4 February in Helsinki) on the proposal to include EE2 and diclofenac to the list of HELCOM coreset indicators.

Larsson gave an invited talk at a seminar on pharmaceuticals in the environment within the series "Pro-et-Contra" arranged by KTH.

Kristiansson presented a talk at the Swedish Bioinformatics Conference in Gotheburg.

February
Larsson gave an invited talk to the Dean and all Prefekts at the Sahlgrenska Academy, University of Gothenburg.

March
Invited presentation by Larsson at the 2nd International Conference on Sustainable Pharmacy, Osnabruck, Germany.

April
Larsson presented a talk at a conference in Stockholm on Pharmaceuticals in the Environment arranged by the Swedish Toxicological Society and the Swedish Toxicological Council.

Larsson gave one of the Keynote talks at the Spring Meeting for Swedish Microbiologists.

May
Larsson was invited by the UK Environmental Research Council (NERC) to give one of the keynote talks at a NEOMICS meeting (NERCs Environmental 'Omics Strategies") in Cardiff and to participate in a workshop with the purpose of identifying national strategies for future research in this area.

Larsson was co-chair for three sessions on Pharmaceuticals in the Environment at the 20th annual SETAC Europe meeting in Seville. The Larsson group also gave, in total, five presentations.

Larsson was selected, as one of only three scientists, to present ongoing research to about 30 of the key staff from the Department of Social Affairs on a one-day visit at Gothenburg University.

Larsson gave an invited talk at a seminar for mainly politicians in the Västra-Götaland region focused on pharmaceuticals in the environment.

Jes la Cour Jansen. Future wastewater treatment in Sweden. Meeting arranged by IDAmiljø, 4. May 2010.

June
Larsson served as external reviewer for a PhD-viva on estrogens in fish at the University of Exeter, UK.

Larsson gave an invited talk at the AstraZeneca, Brixham environmental laboratory in the UK.

Larsson gave an invited talk at the yearly meeting for the "Occupational society for health and environment", a society for staff employed by the municipalities in Sweden.

Henrik R. Andersen. Removal of pharmaceuticals from wastewater. Advanced Urban Wastewater Treatment 1-3 juni 2010. Course for engineers. Arranged by LU/DTU and Svenskt Vatten.

Anna Ledin. Xenobiotics in wastewater and sludge. Advanced Urban Wastewater Treatment 1-3 Juni 2010. Course for engineers. Arranged by LU/DTU and Svenskt Vatten.

July
July 2010. The Larsson group presented three papers at the FEBS 35th Congress with abstract published in the FEBS journal FEBS stands for "Federation of the Societies of Biochemistry and Molecular Biology".

September
Larsson presented an evening lecture for the "Gothenburg Medical Doctors Society".

Cuklev, Kristiansson and Larsson gave three presentations on MistraPharma-related research at meeting for the strong research platform/network "Ecotoxicology- from gene to Ocean" in Gothenburg.

Larsson took part in arranging an open "Cafe-evening" at Ekocentrum in Gothenburg, also presenting a talk on "Cheap medicines – to what price?"'

October
Larsson gave an invited keynote lecture on "Dermatology and the environment" at the 19th Congress of the European Academy of Dermatology and Venereology, in Gothenburg 2010.

October 2010. Larsson gave one of the talks at a seminar on environmental and ethical aspects on the procurement of medicines, arranged by Stockholm County Council.

Larsson gave one presentation in a seminar on pharmaceuticals in the environment at the Karolinska University Hospital.

November
Larsson took part in the arrangement of a seminar on "global antibiotic resistance" within "global week" in Gotheburg. Larsson gave one of three talks.

Per Falås. Pharmaceutical Reduction in Swedish sewage treatment plants.. Envisys Ny teknik seminar. 17–18 November. Lund.

December
Kristiansson took part in arranging a conference on "Deep Sequencing Techniques -Bioscience Applications, Statistical and Computational Practice and Challenges" at Chalmers University of Technology, Gothenburg. Johnning presented one talk, and we presented several posters.

MistraPharma researchers 2011

January
Jes la Cour Jansen. How shall we deal with pharmaceuticals from the toilet? Seminar arranged by MistraPharma in collaboration with Svenskt vatten - "From tablet to the toilet". 18 January 2011. Stockholm.

February
Larsson gave the keynote talk at the yearly "Läkemedelsriksdagen" in Stockholm. Among the other speakers, were, for example, the Swedish Minister of Social Affairs, the State Secretary of Social Affairs, the General Directors of the Medical Products Agency (LV), the National Board of Health and Welfare (Socialstyrelsen), the Dental and Pharmaceutical Benefits Agency (TLV) and the Swedish Council on Health Technology Assessment (SBU) and many others.

Larsson gave, as one of three selected Swedish environmental scientists, a presentation on our work for the Swedish Minister of the Environment and his closest staff at their visit to the FORMAS Research Council.

Henrik R Andersen. Wastewater from Hospitals. Seminar "Sustainable Hospitals" - wind turbines on the roofs? Christiansborg 24. February 2011

March
Larsson presented a talk within the open lunch seminar series of the Sahlgrenska Academy.

Larsson presented the first talk at the newly started seminar series within the "Centre for Global Health" at the Sahlgrenska Academy.

Larsson is co-chair of two sessions at the upcoming SETAC Europe meeting in Milan in May.

Jes la Cour Jansen. Membrane bioreactor for wastewater treatment. MEMBRANDAG 2011. 8 March 2011, Lund

November
Larsson will be one of the keynote speakers at Läkemedelskongressen "The Pharmaceutical congress" in Stockholm.

Staff

Karin Liljelund (Stella Futura), Helene Hagerman (Trossa AB) and Vendela Roos (KTH).