MistraPharma

Annual report 2012

Annual report for MistraPharma 2012

After a successful application and review process MistraPharma was granted funds for an additional four year phase (2012-2015). We are incredibly grateful for the prolonged funding and the confidence and appreciation that Mistra has shown our achievements, and we look forward to continue the work within MistraPharma during this second four-year phase.

Major novelties in the programme during phase 2 are two new partners: First, Brunel University represented by professor John Sumpter. Professor Sumpter contributes world leading expertise in the area of aquatic ecotoxicology and an international network in the field of pharmaceuticals in the environment. And second, KTH Bioprocess Technology represented by professor Gen Larsson and Berndt Björlenius. KTH Bioprocess Technology replaces Lund University and brings the expertise necessary to take new waste water treatment technologies closer to implementation.

Furthermore, we brought in the issue of antibiotic resistance caused by environmental level of antibiotics as a novel research question. This is pursued by Joakim Larsson at the university in Gothenburg. Antibiotic resistance is a global health concern and generating novel information about these processes is of vital interest to our stakeholders.

Our efforts to identify and test pharmaceuticals of environmental concern continue and the work to synthesize the findings of MistraPharma is being intensified. During 2012, the program and several of its stakeholders contributed to the Swedish All-Party Committee on Environmental Objectives, where pharmaceuticals in the environment was one of three prioritised areas.

The interim report from the Committee to the Swedish Government – Reducing the risk of hazardous substances (SOU 2012:38) – proposes the following milestone target for pharmaceuticals:

"Sweden's efforts shall contribute to decisions being taken that lead to environmental aspects being considered in existing and, where necessary, new regulatory frameworks concerning pharmaceuticals, no later than 2020".

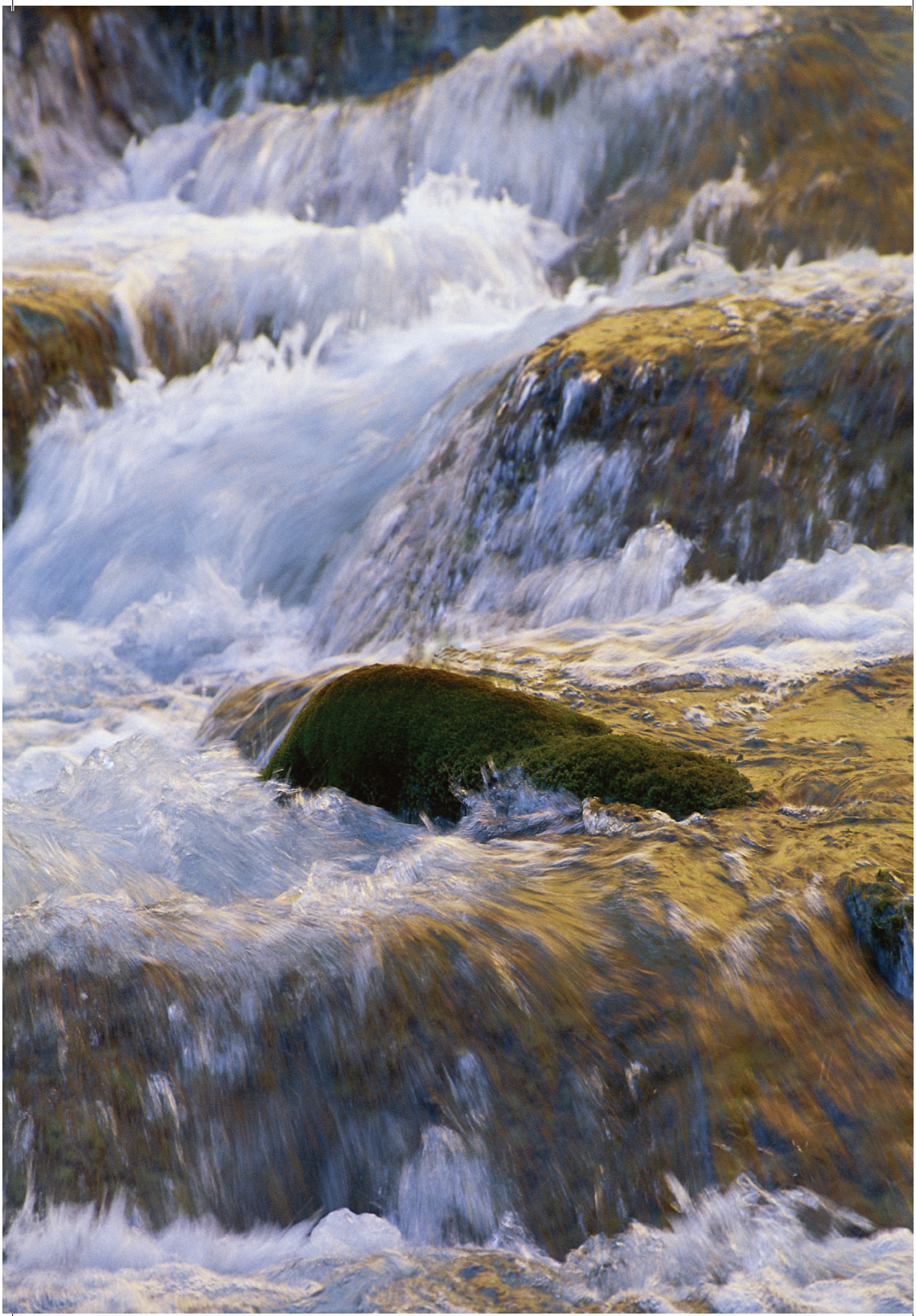
Towards this target the following measures are proposed:

- Introduction of scope to consider environmental aspects in the benefit-risk assessment carried out as part of the authorization procedure for medicinal products for human use
- Introduction of environmental requirements into the European Standards on Good Manufacturing Practice (GMP)
- Introduction of more stringent testing requirements for medicinal products and better environmental risk assessments in accordance with EMA guidelines
- Creation of a database at EMA to collect environmental data on active pharmaceutical substances and make these data available.

MistraPharma look forward to support the development of these proposals together with our stakeholders.

Finally, I would like to express sincere gratitude to our committed Program Board and Reference Group; *Your continuous support and excellent input to the program is extremely valuable and highly appreciated, Thank you!*

Christina Rudén, programme director



Content

Programme structure

Programme Board

Chair:
Charlotte Unger, the Medical Products Agency

Other members:
Berit Balfors, KTH (until the 7th of September 2012)
Åke Bergman, Stockholm University
Nina Cromnier, Swedish Chemicals Agency
Bengt Mattson, LIF
Lena Söderberg, Swedish Water & Wastewater Association
Mikael Hoffmann, Stiftelsen Nätverk för läkemedelsepidemiologi (from the 7th of September 2012)

Co-opted members:
Christopher Folkeson Welch
Karin Liljelund
Christina Rudén

The board has held three recorded meetings during the period (120312, 120907, 121114).

Programme director

Christina Rudén

Commmunication manager

Karin Liljelund

Reference group

Alicja Andersson, Medical Products Agency
Camilla Berglund, Dental and Pharmaceutical Benefits Agency
Annika Christensson, Blekinge County Council
Per Ola Darnerud, National Food Administration
Agneta Edberg, The Association for Generic Pharmaceuticals/Mylan
Anders Finnson, Swedish Water & Wastewater Association
Jerker Forsell, Ministry of the Environment
Linda Gårdstam, Swedish Environmental Protection Agency
Britta Hedlund, Swedish Environmental Protection Agency
Gisela Holm, LIF/AstraZeneca
Lars Lööf, Västmanland County Council
Inger Näsman, Swedish Pharmacy Association/Kronans Droghandel
Therese Olsen Ström, Uppsala University Hospital
Marie-Louise Ovesjö Håkansson, Södersjukhuset AB
Nicklas Paxéus, Gryaab AB
Stephan Quittenbaum, Kronoberg County Council
Per Rosander, International POPs Elimination Network (IPEN)
Karin Tegmark-Wisell, Swedish Institute for Communicable Disease Control
Nina Viberg, Swedish Association of Local Authorities and Regions
Cajsa Wahlberg, Stockholm Water
Ann-Sofie Wenersson, Swedish Agency for Marine and Water Management

Webpage and contact

www.mistrapharma.se

MistraPharma
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Financial Report

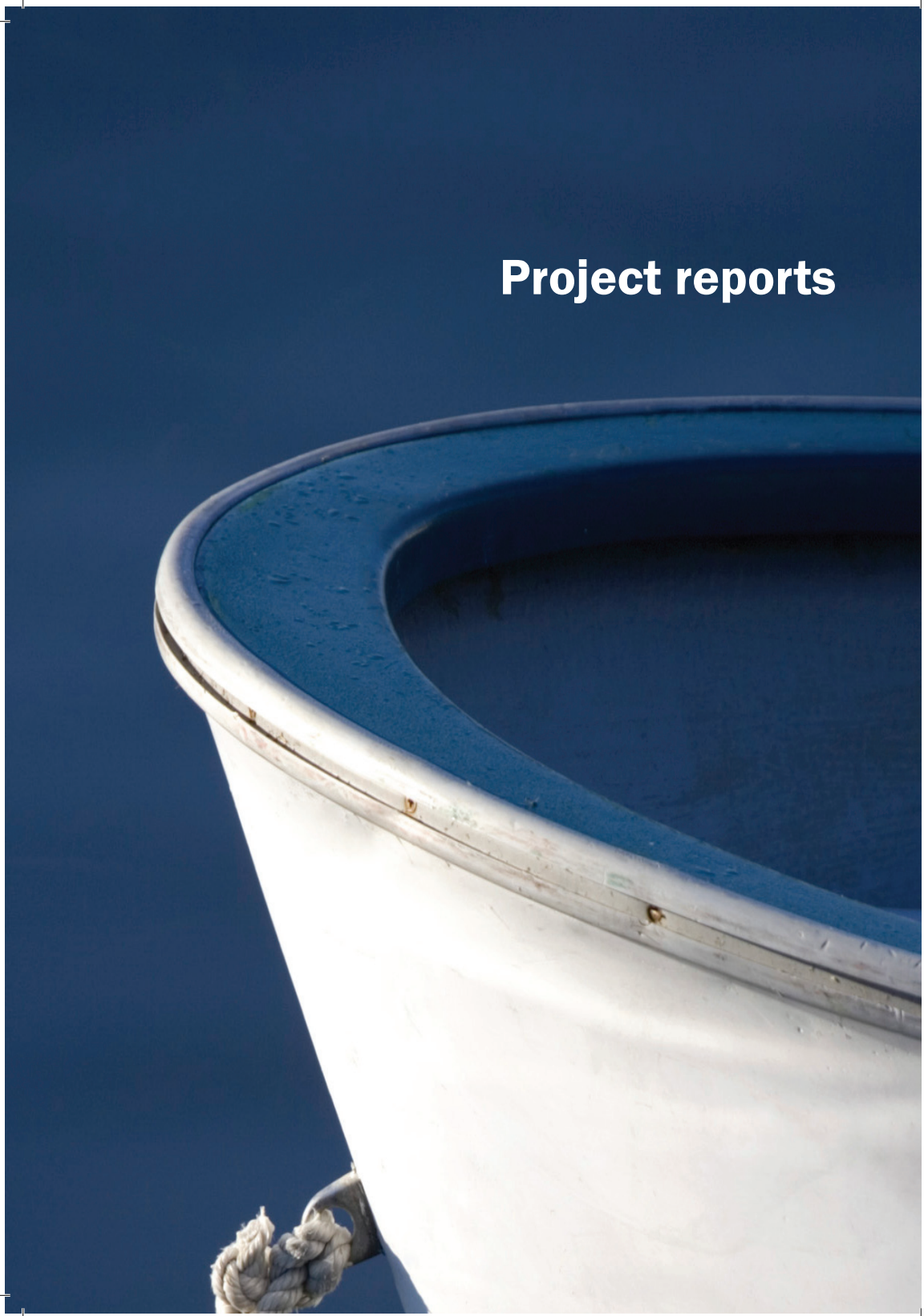
	Outcome 2012
REVENUES	
Allocated funding from Mistra	11 483 244
Other revenues*	1 093 306
TOTAL REVENUES	**12 509 391**
COST	
Personnel costs	4 699 865
Travel expenses	178 523
Supplies	678 982
Depreciation	23 986
Other operating expenses	404 122
DIRECT COSTS	**5 985 478**
Overhead including premises costs	1 117 356
Costs including overhead	**7 102 834**
Purchased services	773 373
TOTAL COST	**7 876 207**
BALANCE	**4 633 184**
ALLOCATED FUNDS PHASE 2 2012	**11 483 244**
REMAINING FUNDS	**40 516 756**

* Stockholm University (183 306 SEK) - still not transfered to Brunel University (pertains to the last four month period, 2012).

* Umeå University (860 000 SEK) - refers to additional funds for analytical support and methodology development.

* Communication project (50 000 SEK) - refers to additional funds from LIF.

Project reports



Umeå University

Project leader: Mats Tysklind

Summary of completed research 2012

Evaluation of High Risk APIs

Identification and evaluation API's of high concern has continued during 2012. This work was based on the fish plasma model, i.e. calculations based on the human therapeutic plasma concentration, usage statistics (PEC) and theoretical bio concentration, which yields an effect ratio (ER). Our extended bio concentration study that was performed in 2009 at Gryab (Gothenburg) and downstream the sewage treatment plant in Skövde has now been analyzed, including tissue distribution by including samples of muscle, brain, liver and bile taken simultaneously. Two manuscripts are in preparation, one that focus on the fish plasma model and the implications this model has on regulatory processes and one focusing on the tissue dependent bio concentration. In addition to the controlled BCF-studies at waste water treatment plants, samples of fish plasma from caught wild fresh water fish in the UK and Germany has been analyzed. Results from this study were presented at the EuroTox 2012 in Stockholm and a full manuscript is in preparation.

As a measurement of persistency of API's, photo degradation experiments have been conducted using a spiking procedure of a mix of API's to different water systems. In these studies the photo degradation of 90 APIs were investigated in three different conditions (pure MillliQ water, a typical Northern European River and a typical Central European River) and at natural as well as artificial sunlight. The data indicate not only different degree of photo degradation dependent of chemical structure of the API but also differences dependent on the water composition. A manuscript is in preparation presenting these new data.

Antibiotic Resistance

Field studies of antibiotic residues in effluent and sludge from operating, full-scale Swedish sewage treatment plants have been initiated and are ongoing. Field studies of river waters and sediments at productions sites and reference sites in heavily polluted areas in Pakistan have been performed. As a result, two papers addressing the environmental levels required to

promote antibiotics resistance was submitted as well as one paper on the environmental levels of antibiotics and associated ARGs in Pakistan. These three papers and a method paper were included in doctoral thesis of Ghazanfar Ali Khan, Umeå University, 2012.

Removal of prioritized APIs in Waste Water Treatment

Results from chemical oxidation experiments with ozone and hydrogen peroxide during MistraPharma Phase I (LU and DTU) have been evaluated and several papers submitted for publication. Evaluation of additional ozone treatment experiments has been initialized. Planning of activities in Phase II.

Analytical determinations

The development of improved screening methods for determination of +100 APIs has been continued. At the same time efficient targeted analyses has been improved. Specific efforts has been taken to establish sensitive and extended analytical methods for progestines. The analytical sensitivity has been optimized in all steps, from sample handling, sample clean-up to optimization on different analytical instrumentation reducing the LOQs with a factor 10-100. Method development for progestines will continue during 2013. In addition, improved analytical methods for particle phase API's has been performed and QA/QC protocols for new API's have been established. Analytical support has been given all partners of MistraPharma.

Regulatory risk assessment and management

Data mapping and modelling have been performed to support the concepts of bio concentration and persistency in the risk assessment and management of APIs

Plans for 2013

The studies of photo transformation/degradation will continue including search for transformation products. Environmental (matrix) factors of importance for photolysis will be investigated. Several manuscripts will be finalized concerning bio concentration. Of special interest is the concept of the fish plasma model and the implications this model has on regulatory processes. Data mapping of results into the physico-chemical map of API's will be conducted as well as attempts to establish QSARs and QSPRs based on biological as well as physic-chemical features (e.g. oxidations rates, photolysis half-lives etc.). Further, special activities will be directed to the occurrence and exposure of progestine as well as antibiotics in STP's.

Further during 2013, Umeå University will continue to support Mistra-Pharma will high quality analytical support. This include, support in exposure set-up and verification of doses in biological test systems and coordination and QA/QC of standard substances. Special efforts will be taken to support the extensive testing and evaluation of tertiary waste water treatment technologies. Additional method development and improvements will be performed to meet the demands of the program.

Staff 2012

Jerker Fick, Richard Lindberg, Marcus Östman, Patrik Andersson, Hanna Söderström, Ghazanfar Ali Khan, Roman Grabic, Katarina Grabicova, Oksana Golovko and Mats Tysklind.

Publications 2012

Published manuscripts

Grabic R, Fick J, Lindberg RH, Fedorova G, Tysklind M. 2012. Multi-residue method for trace level determination of pharmaceuticals in environmental samples using liquid chromatography coupled to triple quadrupole mass spectrometry Talanta 100, 183-195.

Cuklev, F., Fick, J., Cvijovic, M., Kristiansson, E., Förlin, L., Larsson, D.G. J. 2012. Does ketoprofen or diclofenac pose the lowest risk to fish? Journal of Hazardous Materials, 29, 100-106.

Khan GA., Grabic R., Fick J. 2012. Method development and validation of on-line coupling of solid-phase extraction to liquid chromatography-tandem mass spectrometry for the simultaneous determination of anti-infectives and nasal decongestants. Journal of Pharmaceutical and Biomedical Analysis 66, 24-32.

Roos, V., Gunnarsson, L., Fick, J., Larsson, D.G.J., Rudén, C. 2012 Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Science of the Total Environment 421, 102-110.

Säfholm M, Norder A, Fick J, Berg C. 2012. Disrupted oogenesis in the frog Xenopus tropicalis after exposure to environmental progestin concentrations. Biology of Reproduction 86(4)

Hey, G., Grabic, R., Ledin, A., Jansen, J. la Cour., Andersen, H. R. 2012 Oxidation of pharmaceuticals by chlorine dioxide in biologically treated wastewater Chemical Engineering Journal, 185, 236-242.

Breitholtz M, Näslund M, Stråe D, Borg H, Grabic R, Fick J. 2012. An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants. Ecotoxicology & Environmental Safety 78, 63-71.

Manuscripts in preparation/submitted

Khan GA, Berglund B, Khan KM, Lindgren PE, Fick J. Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities − a study in Pakistan. Submitted manuscript

Hey G, Vega SR, Fick J, Tysklind M, Ledin A, la Cour Jansen J, Andersen HR. Removal of pharmaceuticals in WWTP effluents by ozone and hydrogen peroxide. Submitted manuscript

Antoniou MG., Hey G, Vega SR, Spiliotopoulou A, Fick J, Tysklind M, Ledin A, la Cour Jansen J, Andersen HR. Ozone dose for removing pharmaceuticals from wastewater effluents. Submitted manuscript

Doctoral thesis

Khan, G. A., Monitoring anti-infectives and antibiotic resistance genes - with focus on analytical method development, effects of antibiotics and national perspectives, Doctoral thesis, Umeå University, 2012. ISBN 978-91-7459-531-4.

Publications associated projects

Berglund B, Khan GA, Weisner SEB, Ehde PM, Fick J, Lindgren PE. Exposure of constructed wetlands to environmental concentrations of antibiotics shows no effect on antibiotic resistance gene selection and expression. Submitted manuscript

Khan GA, Berglund B, Weisner SEB, Ehde PM, Lindgren PE, Fick J. At environmentally-relevant concentrations, antibiotics do not affect bacterial community patterns in constructed wetlands. Submitted manuscript

Brodin T, Fick J, Jonsson M, Klaminder J. 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations Science 239, 813-814.

University of Gothenburg and Chalmers University of Technology

Project leader: Joakim Larsson (University of Gothenburg)
Assistant project leader: Erik Kristiansson (Chalmers University of Technology)

Summary of completed research 2012

Between January 2012 and now, the Gothenburg team published 6 original research articles on pharmaceutical in the environment, 4 reviews or book chapters and presented a large number of papers at scientific conferences (not listed). In addition we have preliminary results for more than a handful of papers in preparation. Here we highlight a few of the published an ongoing studies.

In 2012 we investigated how fish were affected by exposure to differently treated sewage effluents, using both advanced, explorative methods, i.e. gene expression microarrays, as well as measurements of their relative organ weight (Cuklev et al, 2012a). Exposure to conventionally treated effluent caused a significant increase in the relative size of the liver and heart, an effect removed by all other treatments investigated. Genes connected to xenobiotic metabolism were induced in fish exposed to conventionally treated effluents, though only effluent treatment with granular activated carbon or ozone at 15 mg/L completely removed this response. The RNA expression of heat shock protein 70 kDa was induced in all three groups exposed to ozone-treated effluents, even the low dose (5 mg/L), suggesting some form of added stress in these fish. The induction of estrogen-responsive genes in fish exposed to conventionally treated effluent was effectively reduced by all investigated advanced treatment technologies, although the moving bed biofilm reactor was least efficient. Taken together, granular activated carbon showed the highest potential of reducing responses in fish induced by exposure to sewage effluents.

In another study, we showed that ketoprofen is taken up in fish considerably less than another NSAID, diclofenac. Even at concentrations much higher than those encountered in the Swedish environment, we found no clear physiological responses in exposed fish (Cuklev et al, 2012a). However, a comparison with data on bioconcentration of NSAIDs in effluent-exposed

fish suggests that exposure experiments with single drugs in pure water tend to underestimate uptake/bioconcentration, and consequently the risks for effects. This complicates the interpretation of lab exposures and possibilities to extrapolate to the field situation. The implications of these findings potentially has very far reaching consequences for risk assessments, thus we have chosen to conduct follow-up studies during 2013.

We also investigated how rats (as models for humans and other terrestrial vertebrates) are affected by an acute (5 day) oral exposure to effluent from Indian drug manufacturing (Rutgersson et al, in press). Despite a comprehensive explorative approach, we could not find any effects at all. In several earlier studies we have demonstrated strong effects on water-breathing fish and frogs. By analysing drug residues in the blood of the rats, we could conclude that the lack of effect in the rat is most likely due to a limited internal exposure (rat drink water, fish and frogs breathe it!). We can, however, not rule out that higher doses of effluent or a longer exposure time may still be associated with risks for terrestrial vertebrates. We are, in parallel, assessing risks for the promotion of antibiotic resistance, an effect not investigated in the rat study.

If we were able to directly compare large datasets on gene expression generated in different species, say fish and rats, it might be easier to identify evolutionary conserved responses. This can be useful in order to identify robust biomarkers. However, due to evolutionary events such as gene duplication, there is no one-to-one correspondence between genes from different species which makes such comparison difficult. We recently published a novel statistical method that allows such comparisons (Kristiansson et al, in press). We applied it to estrogen-exposed fish from different species and were able to identify both known and previously neglected responses.

In 2012 we sampled incoming and outgoing effluent, primary, activated and digested sludge from three full-scale sewage treatments plants to study what antibiotic resistance genes are present and how their frequencies change during the treatment processes. The number of culturable bacteria was reduced, as expected, between in and outlet. Analyses of resistance genes will be completed in 2013. Methodologically, we have been working on the construction of an in-house resistance gene database. We have also been able to implement a method to stitch the millions of short DNA reads generated from the DNA sequencing together to longer sequences. This is important in order to identify the resistance genes with greater certainty, and longer sequences can sometimes also provide information on the potential for the resistance genes to be mobile, i.e. move from one bacterium to another.

Plans for 2013

We will sequence microbial communities from the treatment plants sampled in 2012 to study resistance genes. We are currently performing experiments to assess how co-exposure to treated sewage effluent affects the pharmacokinetics of an NSAID in fish. We will attempt to complete our ongoing work on bioconcentration and effect studies on corticosteroids as well as flecainide on fish. We will attempt to complete our study where we compare the production site of the API for products on the Swedish market with the price for the final products, placing the results in light of environmental performance and corruption data from the production countries.

Staff

Filip Cuklev, PhD student within MistraPharma, presented his thesis in 2012 and has now received a position at the Genomics Core facility at the Sahlgrenska academy. PhD students Johan Bengtsson and Carolin Rutgersson, researcher Dr Carl-Fredrik Flach and Technician Birgitta Weidegård have been working within MistraPharma part time. Postdoctoral Fellow Lina Gunnarsson started on a 2 year postdoc in Larsson's lab in April 2012. Postdoctoral Fellow Bethanie Carney-Almroth worked in Larsson's lab in 2012, and has now moved to Department of Zoology, where she recently received her own Formas project grant on "A sea of plastic", starting in 2013. Larsson moved in late 2012 his employment from the Institute of Neuroscience and Physiology to the Institute of Biomedicine, both within the Sahlgrenska Academy at the University of Gothenburg. The rest of Larsson's group, as well as his funding, is expected to follow when labs and offices at Biomedicine are ready (in 2013). Erik Kristiansson (Chalmers) became "docent" in 2012 and PhD student Fredrik Boulund in his group was also working part time within MistraPharma. The collaboration between Larsson's group and Prof Michael Axelsson and his group at Zoology was intensified during 2012. Larsson received the prestigious Swedish Fernström Prize 2012 for "Successful research on pharmaceuticals in the environment".

Publications

Original-articles

Cuklev F, Kristiansson E, Gunnarsson L, Cvijovic M, Rutgersson C, Fick J, Grabic R, Björlenius B, Larsson DGJ. 2012a Global hepatic gene expression in fish exposed to sewage effluents: A comparison of different sewage treatment technologies. Science of the Total Environment. 427-428:106-114.

Cuklev F, Kristiansson E, Cvijovic M, Fick J, Förlin L, Larsson DGJ 2012b. Does ketoprofen or diclofenac pose the lowest risk to fish? Journal of Hazardous Materials. 229¬-230:100-106.

Roos V, Gunnarsson L, Fick J, Larsson DGJ, Rudén C. 2012. Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Science of the Total Environment. 421-422: 102-110.

Manuscripts in preparation/submitted

Shanmugam G, Sampath S, Selvaraj KK, Larsson DGJ, Ramaswamy BR. Non steroidal anti inflammatory drugs and acetylsalicylic acid in Indian rivers. Submitted.

Brosche S, Fick J, Larsson DGJ, Backhaus T. Effluents from antibiotic production induce tolerance development in natural freshwater bacterial communities. Submitted, to be revised.

Reviews and book chapters

Larsson DGJ, 2012. Utsläpp från läkemedelsindustri påverkar miljön - Antibiotikautsläpp riskerar också vår egen hälsa. Invited review in Swedish. Läkartidningen, no 14-15, vol 109, pp 750-753. http://www.lakartidningen.se/07engine.php?articleId=18064

Boxall ABA, Rudd MA, Brooks BW, Caldwell DJ, Choi K, Hickmann S, Innes E, Ostapyk K, Staveley JP, Verslycke T, Ankley GT, Beazley KF, Belanger SE, Berninger JP, Carriquiriborde P, Coors A, DeLeo PC, Dyer SD, Gagné F, Giesy JP, Hallstrom L, Karlsson M, Larsson DGJ, Lazorchak JM, Mastrocco F, McLaughlin A, McMaster ME, Meyerhoff RD, Parrott J, Snape JR, Murray-Smith R, Servos MR, Sibley PK, Straub JO, Szabo ND, Topp E, Tetreault GR, Trudeau VL, Van Der Kraak G. 2012. Pharmaceuticals and Personal Care Products in the Environment: What are the Big Questions? Environmental Health Perspectives 120:1221–1229.

Janzon A, Kristiansson K, Larsson DGJ. 2012. Environmental microbial communities living under very high antibiotic selection pressure. Invited book chapter in: Antimicrobial Resistance in the Environment. First Edition. Eds. Montforts HMM, Keen PL. Wiley & Blackwell. Pp 483-501.

Gunnarsson L, Kristiansson E and Larsson DGJ. 2012. Environmental Comparative Pharmacology: Theory and application. In: Emerging Topics in Ecotoxicology, 1, Volume 4, Human Pharmaceuticals in the Environment - Current and Future Perspectives. Eds: B Brooks, D Huggett. Springer Verlag. ISBN 978-1-4614-3419-1. Pp 85-108.

Reports

Larsson DGJ. 2012. Antibiotics in the external environment – a driver of resistance? Invited report to the European Environment Agency, to be included in an upcoming publication from the EEA on ”Emerging chemicals”.

Selected Popular communication

Fagerberg B, Larsson DGJ, Hagström B. 2012. Prispressade läkemedel utan miljöhänsyn kan stå oss dyrt. Medicinsk kommentar, Läkartidningen no 14-15, vol 109, pp 742-743. http://www.lakartidningen.se/07engine.php?articleId=18062

PhD-thesis

Cuklev F. Transcriptomics and bioconcentration studies in fish to identify pharmaceuticals of environmental concern. PhD thesis. 23 March, 2012. University of Gothenburg.
ISBN: 978-91-628-8431-4. E-published at http://hdl.handle.net/2077/28251

Teaching - undergraduates and practioners

Larsson, Gunnarsson, Cuklev and Carney-Almroth have taught "pharmaceuticals in the environment" between 2011 and 2013 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. Teaching has extended to practicing high-school teachers, nurses and medical practitioners at Chalmers University of Technology and the Nordic School for Public Health (NHV).

Uppsala University

Project leader: Ingvar Brandt

Summary of completed research 2012

To determine the effects of norethindrone and progesterone on egg maturation, adult female frogs were exposed for four weeks to 1-100 ng/L of progestins. Ovaries were evaluated histologically to quantify eggs in different stages of maturation. mRNA levels for vitellogenin, and estrogen- and androgenreceptors were recorded. The aromatase activity in ovaries and brain were measured as well. The results suggest that both compounds inhibit egg maturation, particularly the transition from the previtellogenic stage to the stage when vitellogenin is incorporated in the eggs, i.e. the same effect as previously described for levonorgestrel (LNG). The study will be supplemented with additional experiments when data from the chemical analysis is available.

A developmental study combining exposure to EE2 and LNG is under way following different exposure combinations. Animals killed at metamorphosis have been analysed with regard to gonad histology, and hepatic mRNA levels for hormone receptors and vitellogenin. A manuscript is being drafted. Dissections of adult animals have been carried out and data is under analysis. Histological evaluations of the ovaries remains.

Work is in progress to develop suitable endpoints for progestagenic effects on the development of sex organs in frogs. Basal expression of a number of candidate genes will be quantified; some genes have so far been evaluated following short term progesterone exposure.

To evaluate the importance of the androgenic component of LNG in fish, we exposed adult female sticklebacks to different LNG concentrations for three weeks. Spiggin is produced in the kidneys of male sticklebacks and is an estab¬lished biomarker for androgens. Transcript levels of spiggin were strongly induced together with markedly increased kidney weight and epithelial height in the proximal tubules of the kidneys. Vitellogenin transcription was decreased in a dose-dependent manner. This finding implies that reproductive effects by LNG in fish may be due both to a gestogenic and an androgenic effect.

A manuscript on effects of effluent water from drug manufacturing in the

Hyderabad area in India on the cytochrome P450 1 (CYP 1) system in stickleback was finalized and is now published (Beijer et al. 2013). In this paper we measured transcript expression of CYP genes and a number of other genes as biomarkers for exposure. We conclude that the altered function of the CYP system may affect various physiological functions including the regulation of endogenous hormone levels.

Plans for 2013

In light of the strong androgenic effect of LNG in female sticklebacks, a similar exposure was conducted on males. Spermatogenesis is quiescent in male sticklebacks during the breeding season due to the high plasma levels of androgens, but resumes as androgen levels drop. Males were exposed at the end of the breeding season for 60 days. Preliminary results show that LNG inhibited the onset of spermatogenesis at similar concentrations that induced spiggin production in females. Analyses on testis and kidney histology and tissue-specific gene transcription are under way and will continue for the first half of 2013.

The androgenic potency of LNG in female stickleback was shown to be similar to, even exceeding those of model androgens. The same model androgens can cause male-biased sex ratios in zebrafish at low ng L-1 concentrations. An LNG exposure of zebrafish embryos will be conducted to evaluate its effects on sex ratio and other developmental endpoints.

A manuscript on effects of effluent water from drug manufacturing in the Hyderabad area in India on the cytochrome P450 1 (CYP 1) system in stickleback was finalized and is now published (Beijer et al. 2013). In this paper we measured transcript expression of CYP genes and a number of other genes as biomarkers for exposure. A similar biomarker approach will be used to study waste water from Swedish sewage treatment plants following treatment with different technologies. In these studies we will measure responses in rainbow trout directly exposed to waste water treated in different ways in the wastewater treatment plant. We have therefore designed and tested primers for various genes in rainbow trout including various CYP genes, vitellogenin, zona pellucida genes, genes related to oxidative stress etc. The reactions have been optimized and the products sequenced to verify that the right sequences were formed in the reactions. To test responses in rainbow trout and to prepare for the studies at Käppala we have collected differently treated waste water from Henriksdal sewage treatment plant and exposed fish in the laboratory. Together with the team from KTH we have planned for the studies at Käppala and the interior design of the container that will be used in the experiments.

The amphibian facility will be upgraded and a new life-cycle study to examine flutamide or progestins/EE2 will be carried out. Results from a study on developmental effects of combined exposures to a progestin and estrogen will be published. Negative data on male reproductive toxicity of LNG together with assessment of uptake, distribution and target organs binding for LNG in frogs will also be published. A complementary study on reproductive effects of norethindrone and progesterone in adult female amphibians will be carried out, and a paper submitted. A complementary study on endpoints for progestagenic activity in amphibian tadpoles will be carried out. An in vitro method to study effects of APIs on egg maturation in Xenopus tropicalis will be developed.

Staff

Project leader: Ingvar Brandt, Prof

Kristina Beijer, PhD student (sick leave), fish project
Cecilia Berg, Associate professor, frog project
Björn Brunström, Prof, fish project
Kai Gao, PhD student, fish project
Erika Jansson, PhD student, frog project
Maria Jönsson, Assistant professor, fish project
Nick Jindrisek, MSc students, frog project
Anna Mattsson, PhD, frog project
Margareta Mattsson, Research engineer
Liina Navell, MSc students, frog project
Johan Svensson, PhD student, fish project
Moa Säfholm, PhD student (parental leave), frog project
Viktor Tsiamis, MSc student, fish project

Publications

Säfholm M, Norder A, Fick J, Berg C. 2012. Disrupted oogenesis in the frog Xenopus tropicalis after exposure to environmental progestin concentrations. Biology of Reproduction 86; 126: 1-7.
Berg C. 2012. An Amphibian Model for Developmental and Reproductive Toxicity. Methods in Molecular Biology 889:73-83.

Beijer C, Gao K, Jönsson ME, Larsson DG, Brunström B, Brandt I. 2013. Effluent from drug manufacturing affects cytochrome P450 1 regulation and function in fish. Chemosphere 90(3), 1149-57. doi: 10.1016/j.chemosphere.2012.09.023.

Berg C., Backström, T, Winberg, S, Lindberg, R, Fick, J, Brandt, I. 2013. Developmental Exposure to Fluoxetine Modulates the Serotonin System in Hypothalamus. PLoS ONE 8(1):e55053. doi:10.1371/journal.pone.0055053

Svensson J, Fick J, Brandt I, Brunström B. 2013. The synthetic progestin levonorgestrel is a potent androgen in the three-spined stickleback (Gasterosteus aculeatus). Environmental Science & Technology. Printed on line.

Conference presentations

Berg, C, Säfholm M, Fick, J, Norder, A. Environmental progestin concentrations disrupt oogenesis in frogs. SETAC, Berlin, 2012.

Berg, C & Säfholm M. Progestins – potent endocrine disrupters of the female reproductive system. SETAC, Berlin, 2012.

Berg, C, Brunström, B, Brandt, I. Müllerian Duct Dysgenesis - a common cause for female reproductive disorders? Congress of European Societies of Toxicology, EUROTOX 2012.
Säfholm M, Norder A, Fick J, Berg C. Environmental progestin disrupts oogenesis and Müllerian duct development. EUROTOX 2012.

Berg C, Jansson, E, Säfholm M, Olsson, J, Fick J, Brandt I. Combined Exposure to Progestogen and Estrogen Mixtures: Effects on vitellogenin and hormone receptor mRNA expression. 28th Congress European Society for Comparative Physiology and Biochemistry (ESCPB), Spain, 2012.

Säfholm M, Fick J. Berg C, Female specific reproductive toxicity of progestin in amphibians. 28th Congress European Society for Comparative Physiology and Biochemistry (ESCPB), Spain, 2012.

Stockholm University

Project leader: Marlene Ågerstrand

In August 2012 this project moved from the Royal Institute of Technology to the Department of Applied Environmental Science (ITM) at Stockholm University. ITM then also became the program host for MistraPharma.

In December, after receiving her PhD, Marlene Ågerstrand became the project leader of this project. Rudén remains the program manager.

Summary of completed research 2012

Dissertation

On Dec 7th, 2012, Marlene Ågerstrand successfully defended her thesis "From Science to Policy. Improving environmental risk assessment and management of chemicals." The opponent was Professor Andreas Kortenkamp from Brunel University, UK. Members of the evaluation committee were Associate professor Bert-Ove Lund from the Swedish chemicals Agency, Associate professor Henrik Viberg from the Department of Environmental Toxicology, Uppsala University, and Professor Karin Wiberg from the Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences.

Reliability and relevance evaluation of ecotoxicity data for use in environmental risk assessment of chemicals

The work with evaluating the process of evaluation of ecotoxicity data for use in environmental risk assessment continues, a project that we started in 2010. This year, focus has been on improving the usefulness of the criteria an expand their acceptance. Towards this ends we have worked to extend our network, and communicating our results. Together with researchers and risk assessors at the Swiss Centre for Applied Ecotoxicology and the Dutch RIVM we are conducting a ring test of our reliability and relevance criteria. The ring test is divided into two parts and so far over 60 risk assessors from both Europe and North America has completed the first part.

The NORMAN network (www.norman-network.net) invited us to a meeting for a discussion on how our work can be used in their work on emerging environmental substances. We have contributed to their guidance recom-

mendations for how to evaluate reliability and relevance of ecotoxicity data when prioritizing emerging substances. The guidance network is planned to be published in the beginning of 2013.

We were also invited to present our evaluation criteria at a meeting with risk assessors working on Environmental Quality Standards (EQS) from Europe.

We have also presented our results at three conferences: SETAC Berlin, EUROTOX Stockholm and SETAC North America. At the SETAC meeting in North America we met with the chair of SETACs advisory group for Ecological Risk Assessment and planned for a Special Symposium at the SETAC meeting in Glasgow 2013. The title of the session is "Closing the Gap Between Academic Research and Regulatory Risk Assessment of Chemicals".

A spin-off to this project is a parallel process in which we adjust the evaluation criteria to include also other groups of chemicals. Together with researcher at the Danish Technical University (DTU) we are modifying the criteria so that they can be used in risk assessment of nanomaterials. A manuscript is being prepared. We have also had a master student working with evaluation of ecotoxicity and toxicity data for the endocrine disrupting chemical Bisphenol A. The thesis is finalized and we are now re-writing it for publication. This is done with funds from other sources.

Evaluation of the sensitivity of standard vs. non-standard tests for identifying APIs of ecotoxicological concern

This analysis is performed in collaboration with Stockholm University and Gothenburg University. In this project we use the data compiled in the MistraPharma database – WikiPharma – and other sources to compare the sensitivity of standard and non-standard tests for identifying APIs of ecotoxicological concern. This data will be supplemented with information about the APIs' mode-of-action and an analysis of to what extent the observed results depend on the specificity of the effect and endpoint and the conservation of drug targets.

Plans for 2013

In 2013 we will continue working with evaluation of data for use in risk assessment. We will expand the project so that it will include a weight of evidence approach. We will continue the collaborations we have and bring the reliability and relevance criteria closer to implementation by contacts with journals and regulators, participation and organization of international activities such as work-shops and other meetings, and by finalizing the ring test.

We will also plan to write a synthesis paper proposing improved test requirements in the EMA guidelines based on the results in phase 1 Mistra-Pharma. This will be done in collaboration with partners in MistraPharma.

We will also follow up the result of the "Miljömålsberedningen" and provide support to the Swedish MPA.

Staff

In 2012, the following personnel have been directly involved in the project:
- Christina Rudén
- Marlene Ågerstrand
- Magnus Breitholtz

Publications

Roos V, Gunnarsson L, Fick J, Larsson DGJ, Rudén C. 2012. Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Science of the Total Environment 421-422:102-110.

This paper was selected by a committee of the Society of Toxicology Risk Assessment Specialty Section as one of the "Top 10 Best Papers Advancing the Science of Risk Assessment" for 2012.

Doctoral Thesis

Ågerstrand M. 2012. From Science to Policy. Improving environmental risk assessment and management of chemicals. Theses in Risk and Safety from the Royal Institute of Technology. ISBN 978-91-7501-507-1. http://kth.diva-portal.org/smash/record.jsf?pid=diva2:570429

Master Thesis

Edvardsson L. 2012. Reliability evaluation of ecotoxicological and toxicological studies of Bisphenol A. The Royal Institute of Technology.

Stockholm University

Project leader: Magnus Breitholtz

Summary of completed research 2012

A main objective for the Stockholm University project on biological testing in phase 1, but also of major importance for the evaluation of wastewater technologies to be done in phase 2, has been to identify cellular and molecular effects based on the fact that some drug targets in other species than vertebrates (i.e., fish) have also been conserved during evolution. For this purpose, we have exposed Daphnia magna to three selected priority APIs in experiments, where expression of selected genes have been analyzed using qPCR. We have found significant responses in transcription level of genes related to reproduction (vitellogenin) and moulting (cuticle protein). These gene responses have further been related to responses on individual growth (RNA/DNA ratio, body length), heart rate and oxidative status. A manuscript reporting these findings (Furuhagen, Fuchs et al.) was submitted in 2012 and is now in review.

The main focus of the first year of phase 2 has been to develop an oxidative stress-based biomarker approach for a broad-range screening of wastewater treatment technologies. The work coupling oxidative stress and individual growth endpoints that was started in 2011 has been extended and in 2012 we have added lipid peroxidation to our biomarker toolbox. Several studies on the relationships between oxidative stress and physiological performance (i.e., feeding activity) in Daphnia have been performed using e.g. priority API haloperidol as a model substance. The integration between these response variables is central for designing tests in the coming wastewater treatment evaluations. Currently, we are finalizing a manuscript on the outcome of this work (Furuhagen, Liewenborg et al.).

Since the wastewater project at KTH has been delayed, and there has been a need to develop and validate the oxidative stress biomarker-based approach for high throughput testing in the pilot-scale evaluation, we have used wastewaters sampled during the Stockholm Water project. Using waste-water treated with different concentrations of ozone (3-15 mg/L), we have tested our biomarker approach with both daphniids and microalgae as test organisms. Significant efforts have been allocated to optimize experimental designs to be applicable in the coming pilot-scale evaluation. The test system

development and validation have been successful and we are now confident that our approach will be suitable for screening a large number of wastewater samples. These experiments have also shown that there seem to be a threshold around 7-8 mg/L ozone, above which negative effects on antioxidative capacity in both daphniids and microalgae have been observed. This effect is clearly coupled to the ozone concentration used for treating the wastewater, and is potentially a result of formation of reactive transformation products. This finding is certainly of importance for selecting relevant ozone concentrations to be used in the coming pilot-scale evaluation.

As a spin-off project we have also in 2012 studied bacteria-host interactions as a consequence of antibiotic exposure in D. magna. Microbiome is involved in various physiological processes of a metazoan host, such as feeding, immune defence and reproduction. In polluted environments, contaminant effects may be manifested via both direct disturbance of the host physiology and disruption of bacterial communities associated with the host, with concomitant effects on the bacteria-host interactions. In this context, particularly relevant contaminants are antibiotic substances released into environment and affecting both free-living and symbiotic microbial communities. This study is built upon our earlier findings published in 2012 (Edlund et al. 2012) that report dramatic effects of antibiotics (ciprofloxacine, trimethoprime and sulfomethoxazole) on microbiome community in crustaceans. The aims of the present study (Gorokhova et al.) were to understand (1) linkages between feeding capacity of a crustacean host and its associated bacterial communities, and (2) effects of antibiotics on crustaceans in general and D. magna as a model species in ecotoxicology in particular. In the laboratory experiments, effects of the antibiotic drug trimethoprim (0.25-2 mg/L) on composition and abundance of bacterial community associated with D. magna were studied in concert with daphniid survivorship, feeding activity and digestion efficiency. The results showed that both abundance and composition of the bacterial communities were strongly affected by trimethoprim exposure. Quantitative PCR (qPCR) of the gene SSU rRNA revealed a >10-fold decrease in average bacterial SSU rRNA copy number in the exposed daphniids compared to the controls. Moreover, the analysis of clone libraries indicated that bacterial composition has also changed drastically, with a major diversity shift from relatively balanced communities dominated by Curvibacter, Aquabacterium and Limnohabitans groups in the controls to the significantly lower diversity and strong dominance of Pelomonas bacterium in the exposed animals. The feeding in the exposed daphniids fed green algae was severely compromised by the trimethoprim exposure. Significantly decreased clearance rate and food digestion were observedin the exposed D. magna. The clearance rate

decreased with increasing antibiotic concentration in a dose-response manner, being >30% lower in the highest trimethoprim concentration compared to the controls. Application of 14C-labeling technique showed a >40% decrease in assimilation rate in the highest exposure concentration. Moreover, in the exposed daphniids, a significant decrease of dead algal cells in the animal gut was observed, indicating that prey digestion was inefficient. Taken together, these results imply that antibiotics at environmentally relevant concentrations may cause profound effects on non-target eukaryotes via changes in structure and abundance of their microbiomes leading to compromised nutrition, which would inevitably result in decreased growth and population decline. These microbiome-mediated modes of action in eukaryotic non-target animals and plants may not be unique for antibacterial drugs, but also relevant for other environmental pollutants of various nature.

Plans for 2013

In 2013, our main focus will be to evaluate wastewaters from the pilot scale treatment. We will also continue with the work started already in phase 1, i.e. to develop and apply biochemical tools to unravel mechanistic effects in primarily D. magna and to develop and validate population-level mathematical models for improved environmental risk assessment. As this is primarily part of the work the two PhD students involved in our project, a main focus will be on finalizing the manuscripts we have started to prepare in 2012 (in addition to the two above-mentioned manuscripts by Furuhagen et al. also two manuscripts by Lundström et al.).

In phase 1 of the programme, we mainly focused on delivering standard ecotoxicological test data using micro algae (OECD 201), D. magna (OECD, 202, 211 and feeding inhibition test) and fish embryos (OECD 2006) to the synthesis project. Overall, the standard tests have been relatively insensitive to the ca 20 prioritized APIs tested in phase 1. No additional APIs have been tested in the first year of phase 2, but we will do so in 2013 should interesting candidate substances be identified.

Staff

In 2012, the following personnel have been involved in the project:
- Magnus Breitholtz (Associate Professor)
- Elena Gorokhova (Professor)
- Elin Lundström (PhD student)
- Sara Furuhagen (PhD student)
- Birgitta Liewenborg (technician)
- Karin Ek (technician)
- Margareta Linde (technician)
- Pavel Ivanov (Post doc)

Publications

Published manuscripts

Breitholtz M, Näslund M, Stråe D, Borg H, Grabic R, Fick J. (2012) An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants. Ecotoxicology and Environmental Safety 78, 63-71.

Edlund A, Ek K, Breitholtz M, Gorokhova E. (2012) Antibiotic-induced Change of Bacterial Communities Associated with the Copepod Nitocra spinipes. PLOS One 7(3).

Manuscripts in preparation/submitted

Furuhagen S, Fuchs A, Lundström E, Breitholtz M, Gorokhova E. Do pharmaceuticals with evolutionary conserved molecular drug targets pose a greater environmental risk? Submitted to Proceedings of the Royal Society B.

Breitholtz, M., Furuhagen, S., Ek, K., Lindström, K., Ivanov, P., Gorokhova, E. Calmodulin inhibition as a mode-of-action of antifungal imidazole pharmaceuticals in non-target organisms: implications for mixture toxicity assessment.

Gorokhova E, Rivetti C, Furuhagen S, Ek K, Edlund A, Breitholtz M. Microbiome-mediated effects of trimethoprim in Daphnia magna.

Lundström E, Brinkmann M, Breitholtz M, Preuss T. Application of matrix and individual based models in environmental risk assessment.

Lundström E, Preuss, T, Breitholtz M. Validation of an individual based population model with the harpacticoid copepod Nitocra spinipes.

Furuhagen S, Liewenborg B, Breitholtz M, Gorokhova E. Feeding activity and oxidative stress in Daphnia magna.

Conference contributions

Breitholtz M (2012) Ekotoxikologisk utvärdering av avancerade reningstekniker för att ta bort läkemedel från avloppsvatten. Invited speaker at conference: VA-kommunikation: Agera istället för att reagera, FVIT-möte i Tylösand, Sweden, 25-26th of April.

Ågerstrand M, Breitholtz M, Rudén C. (2012) Regulatory perspectives on pharmaceuticals in the environment. Platform presentation at EUROTOX in Stockholm, Sweden.

Ågerstrand M, Breitholtz M, Rudén C. (2012) Standard and non-standard ecooxicity tests in regulatory risk assessment of chemicals. Platform presentation at SETAC 6th World Congress 20-24 May, Berlin, Germany.

Breitholtz M, Näslund M, Stråe D, Borg H, Grabic R, Fick J. (2012) An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants. Poster presentation at SETAC 6th World Congress 20-24 May, Berlin, Germany.

Furuhagen S, Fuchs A, Lundström E, Gorokhova E, Breitholtz M (2012) Do pharmaceuticals with evolutionary preserved drug-targets in non-target organisms pose a greater environmental risk? Poster presentation at SETAC 6th World Congress 20-24 May, Berlin, Germany.

Gorokhova E, Edlund A, Ek K, Breitholtz M (2012) Antibiotic-induced change of bacterial communities associated with the copepod Nitocra spinipes. Platform presentation at SETAC 6th World Congress 20-24 May, Berlin, Germany.

Breitholtz M, Furuhagen S, Ek K, Ivanov P, Gorokhova E (2012) Calmodulin inhibition as a mode-of-action of antifungal imidazole pharmaceuticals in non-target organisms: implications for mixture toxicity assessment. Poster presentation at SETAC 6th World Congress 20-24 May, Berlin, Germany.

Royal Institute of Technology (KTH)

Project leaders: Gen Larsson
Assistant project leader: Berndt Björlenius

Summary of completed research 2012

KTH/Industrial biotechnology became a partner of the MistraPharma project in 2012. The project work started in late April when the assistant project leader was employed. The scientific work began with planning and literature surveys of latest papers published.

Literature showed that ozonation, activated carbon and combination of these processes still have the greatest potential for full scale applications in wastewater treatment. Some attempts to use other adsorbents, than activated carbon, have been reported in literature; Clay and clay-micells can be used at lower cost, but will cause swelling in filters, which prohibit full scale use, until the swelling problem is solved. UV is also discussed a lot in literature but our research shows that the low transmission of UV-light in wastewater and the scaling on the quartz tubes, prevents an efficient use of UV.

During spring and summer a diploma work was done on ozonation and activated carbon filtration. The diploma work was carried out at Henriksdals WWTP (Waste Water Treatment Plant). Lab plants were designed in parallel and built to test variants of ozonation; bubble diffuser and multi stage ozonation.

The pilot plant runs of the ozonation plants showed variations in removal efficiency. The preliminary results showed that multistage ozonation had 10% higher removal than bubble diffuser ozonation. This was so interesting, that the pilot plant was designed with both single stage and multi stage options.

To compare non-activated and activated carbon, charcoals of different wood species were produced by pyrolysis in labscale. Also tests with pyrolysis of digested sludge were performed.

The most promising, in-house produced, carbon types were compared with a well known commercial activated carbon, Chemviron F400. Continuous labtests with three of the granular carbons were conducted in downstream filters.

The adsorbtion capacity of carbons was first characterized by methylene blue number and Iodine number. The in-house carbons had a methylene blue number of 9-23% of F400 and an Iodine number of 30-44% of F400. The Iodine number must be considered as high, thou the carbons are not activated!

The preliminary results from the pilot plant runs showed that the in-house carbons had lower general removal of pharmaceuticals. The removal with in-house carbons varied also much more for different substances. During the five week continuous operation the average removal of pharmaceuticals in per cent was 55-65% for in-house carbons and 92% for the commercial F400.

The chemical analysis of the samples was delayed, so the master thesis could not be finalized in 2012, but in March 2013. (The student got an employment from September 2012)

Based on the diploma work and literature surveys, a selection and detailed design of pilot plants were performed during the autumn. Plants for ozonation and activated carbon plants, in two major forms were designed. Both Granualar Activated Carbon (GAC) and Powered Activated Carbon (PAC) lines will be compared in pilot scale. Two separate lines will be built of both GAC and PAC, so comparisons can be done in parallel of different activated carbon products.

Additionally two plants with biofilm processes were design to continue the evaluation of the positive results from MistraPharma phase 1.

In total 12 different lines have now been designed and blue printed for local production in the workshops at Käppala WWTP. The welding of the reactors started in late 2012. Skilled installation and high quality material is used and so the plant will have long-term durability.

In parallel with the design of treatment processes, a 20 feet container was ordered, insulated and prepared to house the pilot plants. Three, 5 m high ozone columns were prepared for installation in November. Procurements of pumps and on-line meters were performed during late 2012.

A sampling campaign was undertaken in September at three waste water treatment plants of special interest to MistraPharma; Henriksdal, Käppala and Kungsängsverket in Uppsala. These plants will be or have been part of the evaluation in lab or pilot scale tests within MistraPharma. The sampling was a cooperation between KTH and GU. Samples of wastewater were taken from different basins in wastewater treatment, but also samples of sludge from primary, biological excess sludge and digested sludge were taken. The aim of this study is to make mass balances of pharmaceuticals (KTH)

and mapping the occurrence of antibiotic resistant bacteria. Results will be presented in 2013.

Plans for 2013

In early spring 2013 the mobile container lab will be finalized. The pilot lines will be taken in operation gradually. First step optimisation of dose versus performance will be run in April-June. Set-ups for fish exposure test (rainbow trout) will be developed and tested in March-April. Lab plants will then be operated to compare removal in different scales. Sharp exposure tests will be performed in June, when the pilot lines are ready for realistic operation.

During the autumn the mobile lab will be transported to Kungsängsverket WWTP in Uppsala. Similar test like at Käppala WWTP will be performed in Uppsala. The aim of testing at different WWTP is to verify the generality and boundaries of the treatment processes.

Staff

During 2012 the project organization was built up. Professor Gen Larsson is project leader and PhD-student Berndt Björlenius is assistant project leader, executing design and procurements, coordination, daily work planning and trouble shooting for the mobile lab building activities. Planning and evaluation of lab and pilot plant tests are other parts of the assistant role that will be the main focus after finalized building activities.

Jimmy Söderling is process operator, makes purchases of parts to the pilot plants and assists the building activities in the mobile lab. Later he will have the daily operation in his hands.

Louise Jansson, was Diploma worker on ozonation and activated carbon.

Publications

The project was focused on design and mobile lab building activities. No peer review papers were published. Results produced in 2012 will be reported during 2013.

Media showed interest in the project. Representative for the work package was interview in Ny Teknik, Sweden's leading technology newspaper. The media exposure resulted in contacts with several inventors and colleagues in Sweden.

Brunel University

Project leaders: John Sumpter

Summary of completed research 2012

No research was completed in 2012 because Brunel University's contribution to MistraPharma did not get underway until the autumn of 2012 when a new Ph.D. student, Tara Thrupp, began her studies. This delayed start was planned and has not caused any problems whatsoever, with regards to delivering the planned contribution from Brunel University.

Brunel University's contribution to MistraPharma involves assessing the effects of simple mixtures of pharmaceuticals as a first step in trying to understand how complex mixtures of pharmaceuticals might affect fish and other aquatic organisms. The field of mixtures toxicity is not simple, and hence considerable training is required before they are able to contribute in a meaningful way. Hence, Tara spent most of her time in 2012 (after she started in October) learning about mixtures toxicity.

Plans for 2013

We intend to take a step-wise approach. The goal is to conduct some in vivo mixtures experiments, probably involving exposing fish to relatively simple mixtures of steroidal pharmaceuticals. The aim is to determine whether or not the different pharmaceuticals in the mixtures act independently of each other, or interact in some way. Such experiments are difficult to execute successfully, and are also time-consuming and hence expensive. Hence, it is the intention to begin our mixtures toxicity experiments using a simpler experimental protocol. We will investigate the interactions of different anti-cancer drugs, with different mechanisms of action, on the growth of algae. We should be able to learn a lot from those experiments, and use that knowledge to formulate hypotheses which can subsequently be tested on fish. It is anticipated that the work with algae will take six to nine months, enabling the work with fish to begin before the end of 2013.

Staff

Professor John Sumpter
Ms. Tara Thrupp (Ph.D. Student).
Professor Andreas Kortenkamp (the second supervisor of Ms. Thrupp)
Dr Tamsin Runnalls (she is helping to train Ms. Thrupp).

Publications

No publications during 2012.

Communication project

Project leader: Karin Liljelund

The communication project together with the programme researchers are responsible for the external communications towards the main stakeholders. The communication project coordinates the internal communication through programme meetings and monthly telephone conferences.

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

Website

A new website was developed during the year. 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.

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 19 different representatives from our stakeholders. We were very happy to welcome seven new organizations this year; the Dental and Pharmaceutical Benefits Agency, The Association for Generic Pharmaceuticals in Sweden, the Ministry of the Environment, Södersjukhuset AB, the Swedish Institute for Communicable Disease Control, the Swedish Association of Local Authorities & Regions and the Swedish Agency for Marine and Water Management.

The reference group is a vital link to ensure that the outcomes of the program will benefit our stakeholders. In addition to regular contact with the majority of our representatives in the group, following activities was organized during the year:

Meetings

January 31
The meeting was attended by 15 of the reference group's 19 members and MistraPharma program director and members of the communication project.

The purpose of this meeting was to continue to discuss the reference group's expectations and desires of the second phase of the programme. The meeting was very constructive and fruitful. Marlene Ågerstrand presented her research concerning comparison of standard and non-standard tests for risk assessment. All members of the reference group also presented how they see that they contribute to the program and what is going on within their organsiations.

October 23
The reference group meeting was initiated jointly by the researchers' program meeting where the reference group had the opportunity to ask questions and discuss specific issues relating to the research.

The separate reference group meeting aimed to discuss the reference group's needs and desires of communication activities during next year. The meeting ended with a joint dinner with the researchers and the program's board of directors.

Telephone meetings

To further increase the opportunities for interaction between the reference group and MistraPharma telephone meetings were held between regular meetings. 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. A telephone meeting was held the 4th of May with the aim to get input from the reference group on how MistraPharma could continue after its last year, 2015.

Information

Broschure

A new brochure describing the main contributions from phase one of the programme and the overall aims of phase two was developed and printed in the beginning of the year.

Newsletter

During the year two newsletters have been published in English, 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 and are available at our web page. A number of copies have also been printed to be distributed at seminars, conferences and more.

Conferences

MistraPharma participated in the poster exhibition at the Swedish Chemicals Agency conference "Forum för Giftfri miljö" at Münchenbryggeriet in Stockholm the 15th of October.

MistraPharma arranged a workshop "Pharmaceuticals in the environment: occurrence, effects on wildlife, and how to reduce the levels" at the 48th EUROTOX Congress which was held in Stockholm 18-20 of June.

Other communication activities - MistraPharma researchers

University of Gothenburg

The Gothenburg team has engaged extensively in communication activities with stakeholders and the public during 2012. Gunnarsson and Larsson participated in the work coordinated by LIF to develop a new environmental classification system for pharmaceuticals. The purpose of this is to provide means for a possible inclusion of environmental aspects (including pollution from manufacturing) in the prizing system for medicines (under evaluation by the Swedish government). Larsson has participated in two Finnish and two German TV documentaries, and a Swedish TV documentary by Folke Rydén on pollutants in the Baltic Sea. The latter is expected to be broadcasted in the spring or summer 2013. Larsson chaired sessions on pharmaceuticals at both EUROTOX and SETAC Europe and the group presented several talks and posters. Larsson has given a large number of invited lectures, e. g. at: the Finnish pharmaceutical congress, Helsinki, Finland; The Hartmann Insititue, Helsinki, Finland; the conference "In joint battle against Infectious Disease and Antibiotic resistance", Uppsala University; Nationellt Antibiotikaforum, Stockholm and several more. Of particular importance was an invited platform presentation by Lancet Infectious Diseases and the European Society for Microbiology and Infectious Diseases at St Mary's Hospital in London in the honour of Sir Alexander Fleming, the discoverer of penicillin. Larsson also participated in an expert workshop in Canada on antibiotic resistance in the environment.

Stockholm University

Project leader: Marlene Ågerstrand

Christina Rudén has during 2012 had two commission of trust. She chaired an expert group within ”miljömålsberedningen” about pharmaceuticals in the environment. Proposing policy objectives under the governmental environmental objective “A non-toxic environment”.

She was also appointed a member of ECHA’s Management Board.

Rudén has also made presentations of MistraPharma to ITM’s Board, Presentation at Globe Forum, for the organization “Hållbara hav” at “Briggen Tre Kronor ”, Presentation at “Medicinska Riksstämman”, Presentation of MistraPharma to Mistra’s Board, Presentation at EEA Emerging Contaminants meeting, Interviews in various media (Radio, Svenskt Vatten, Ny Teknik, Fokuniversitetet, Mistras nyhetsbrev), Chairing a session on pharmaceuticals in the environment at EUROTOX in Stockholm.

Project leader: Magnus Breitholtz

In addition to six international conference contributions in 2012, Magnus Breitholtz was also invited to present the work related to wastewater treatment technologies within MistraPharma at a national workshop organized by FVIT (“VA-kommunikation: Agera istället för att reagera, FVIT-möte i Tylösand, Sweden, 25-26th of April).

Staff

Karin Liljelund and Helene Hagerman (Trossa AB)