MistraPharma

Annual Report 2014

Annual report for MistraPharma 2014

The year 2014 was another productive year for MistraPharma!

The question of pharmaceuticals in the environment is increasingly discussed at the policy level and now this discussion has also reached the EU level. There are currently two processes of importance for the issue within the EU. The first one is the development by the Commission of a strategic approach to pollution of water by pharmaceutical substances. This process is initiated by a new directive on priority substances in the field of water policy. The second one is the ongoing negotiations in the Council and the European Parliament on veterinary medicinal products and medicated feed. The aim of the process is to increase the availability of veterinary medicinal products while addressing the public health risk of antimicrobial resistance. The latter aspect is closely related to environmental concerns. In both processes, it will be of utmost importance to take into account current scientific knowledge on the environmental effects posed by active pharmaceutical ingredients.

To feed in to these processes MistraPharma researchers, coordinated by our ***Synthesis project at Stockholm University***, have summarized the results from the program and presents ten recommendations for improvements of the European Medicines Agency's guidance for environmental risk assessment of human pharmaceuticals. The manuscript has been submitted for publication in an international peer reviewed journal and is currently under review. Work is ongoing with the aim to be allowed to present these recommendations in the European Parliament later this year.

In addition to this, all other projects have contributed with significant new achievements. Some of the major outputs are summarized below:

Our project for ***chemical analyses in Umeå*** is a fundament and prerequisite for the success of MistraPharma. This year they have analyzed more than 1800 samples! In addition the Umeå scientists have developed a new and very sensitive analytical method for progestins, estrogens and androgens (LOQ at 0.01 ng/L).

Our team at Uppsala University has demonstrated that the progestagen levonorgestrel exerts strong androgenic effects in fish at environmentally relevant concentrations. These effects are manifested e.g. as a reduced ability of male fish to resume spermatogenesis by leaving the reproductive cycle. This effect is likely to be of great ecological relevance.

In addition, the three progestogens levonorgestrel, norethindrone and progesterone were shown to inhibit egg production (oogenesis) in frogs at, low nanogram concentrations. The progestagens are of particular concern given their wide-spread use and the fact that several of them affect the same target, i.e. egg production at low concentrations. This indicates a risk for adverse effects on fertility in exposed, wild amphibians.

The team at Brunel University has carried out a multicomponent mixture study using five steroidal pharmaceuticals in a well-established fish reproduction assay. The purpose of the study was to assess the potential for combination effects on an ecologically relevant end point – reproduction.

Our team at KTH has run the waste water treatment pilot plant at several locations during 2014; After the first four months of operation in Käppala, the pilot plant has visited Uppsala and Västerås, and on December 15th it was moved back to Käppala for winter storage. One reason for moving the pilot plant was to study the effect of waste water composition and type of treatment on the treatability of pharmaceutical residues. The most obvious result, showing just after a few days, was that without a final sand filter at the treatment plant, the up-time for granular activated carbon filters (GAC) was limited.

Work has also started to design Sweden´s first full-scale ozonation treatment step to remove micropollutants. The ozonation step is now under construction in Knivsta.

The ecotox team at Stockholm University evaluates new treatment technologies with ecotoxicological approaches. So far, tested effluents from Käppala and Uppsala have induced relatively weak toxicity in tests with both algae and crustaceans. Preliminary results show that while activated carbon and sand filtration have little impact on effluent toxicity, treatment with ozonation reduces toxicity to both Daphnia and micro algae.

Gothenburg University and Chalmers have shown that bacteria from an antibiotic-contaminated lake in India contained unprecedented numbers of resistance genes for basically all classes of antibiotics. The results also suggested an exceptional adaptation of the bacteria to exchange genes with each other, which clearly enhances the risks for resistance gene transfer to human pathogens.

Our project for stakeholder communication made awareness of MistraPharma research through seminars during Almedalen week, arranged together with among others the Sustainable Seas Initiative, the Chemicals Agency, the Medical Products Agency and Stockholm University. The project have continued to lead the work within the committee appointed to formulate a proposal on how *pharmaceuticals and the environment* can continue after 2016-2020. The Board adopted the proposal of the committee during the spring.

MistraPharma's Board and Reference group have also made crucial contributions to the program. Their continuous input help to ensure that the science performed is relevant and useful for our stakeholders. The combined effort of the entire MistraPharma network of scientists and stakeholders is a key to the success of the program.

Christina Rudén, programme director

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Programme structure

Programme Board

Chair:
Charlotte Unger, the Medical Products Agency

Other members:
Åke Bergman, Stockholm University
Nina Cromnier, Swedish Chemicals Agency
Bengt Mattson, LIF
Anna Linusson replaces Lena Söderberg, Swedish Water & Wastewater Association
Mikael Hoffmann, Stiftelsen Nätverk för läkemedelsepidemiologi

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

The board has held four recorded meetings during the period (140310, 140509, 140915, 141110).

Programme director

Christina Rudén

Commmunication manager

Karin Liljelund

Reference group

Alicja Andersson, Medical Products Agency
Fredrik Nilsson, 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
Lina Wendt - Rasch replaces Kerstin Gustafsson, , the Swedish Chemicals Agency
Linda Gårdstam, Swedish Environmental Protection Agency
Britta Hedlund, Swedish Environmental Protection Agency
Karin Boström replaces Gisela Holm, LIF
Lars Lööf are no longer participating in the group, Västmanland County Council
Robert Svanström replaces Inger Näsman, Swedish Pharmacy Association
Therese Olsen Ström, Uppsala University Hospital
Marie-Louise Ovesjö Håkansson, Södersjukhuset AB
Nicklas Paxéus, Gryaab AB
Per Rosander, International POPs Elimination Network (IPEN)
Sara Byfors, Swedish Institute for Communicable Disease Control
Nina Viberg are no longer participating in the group, Swedish Association of Local Authorities and Regions
Cajsa Wahlberg, Stockholm Water
Ann-Sofie Wenersson, Swedish Agency for Marine and Water Management
Pauline Göthberg, Stockholm County Council

Webpage and contact

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Financial Report

	Outcome 2014	Outcome 2012-2014
OPENING BALANCE*	6 483 423	
REVENUES		
Allocated funding from Mistra	13 729 734	40 614 083
Other revenues**	40 450	1 408 808
TOTAL REVENUES	**13 770 184**	**42 022 891**
COST		
Personnel costs	9 128 732	20 735 840
Travel expenses	548 011	969 153
Supplies	2 137 753	5 205 355
Depreciation	293 071	435 750
Other operating expenses	691 171	1 710 531
DIRECT COSTS	**12 798 738**	**29 056 629**
Overhead including premises costs	2 105 680	4 747 871
Costs including overhead	**14 904 418**	**33 804 500**
Purchased services	1 268 170	2 685 987
Extra transfer***	979 700	979 700
TOTAL COST	**17 152 288**	**38 726 493**
BALANCE 2014	**-3 382 104**	
CLOSING BALANCE	**3 101 320**	
ALLOCATED FUNDS PHASE 2 2012-2015		**40 401 083**
REMAINING FUNDS		**11 385 917**

* Unspent funds as of 2014-01-01.

** Refers to additional funds from LIF (communication project - 50 000 SEK) and loss due to currency transaction (Total 9636 SEK)

*** Umeå University (198 000 SEK), University of Gothenberg (134 000 SEK) and the communication project (47 700 SEK) - from unforseen. Umeå University (600 000 SEK) from analytical determination.

Project reports

Stockholm University

Project leader: Marlene Ågerstrand

Summary of completed research 2014

Suggestion for improvement of the EMA-legislation

Together with researchers from all other work packages we have written a paper presenting ten recommendations for improvements of the European Medicines Agency's guidance for environmental risk assessment of human pharmaceutical products. The recommendations are based on up-to-date, available science in combination with experiences from other chemical frameworks such as the REACH-legislation for industrial chemicals. We believe that implementation of our recommendations would strengthen the protection of the environment and be beneficial to society. The manuscript has been submitted for publication in an international peer reviewed journal and is currently under review.

Systematic review and evidence integration

Together with Swetox and more than 25 experts from Europe and North America we are developing a framework from systematic review and evidence integration for endocrine disrupting chemicals (EDCs). The aim of this effort is to help risk assessors make use of a wider range of data when performing risk assessments. This is especially important for EDCs since standard approaches may not be sufficient for assessing endocrine disruption. Two three-day workshops on the theme "Future directions for application of transparent, consistent and systematic framework for risk assessment of endocrine disrupting chemicals" has been organized at Brunel university in London (in June 2014 and in Jan 2015) and two manuscripts are in preparation. The workshops were executed with additional funds from Mistra.

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

Within the CRED-project (Criteria for Reporting and Evaluating ecotoxicity Data) we have, together with researchers and risk assessors at the Swiss Centre for Applied Ecotoxicology, RIVM, and EAWAG conducted a "ring test" and developed an evaluation method and reporting recommendations for ecotoxicity studies. The ring test showed that the CRED evaluation

method was the preferred method and that it resulted in more transparent and consistent evaluations compared to the method currently in use (the Klimisch method). The project is close to being finalized and four manuscripts are submitted/in preparation.

Plans for 2015

We will continue working with evaluation of studies for use in risk assessment, focusing on the framework for systematic review and evidence integration. We will also organize a SETAC Pellston workshop on the topic. Together with Erik Kristiansson at Chalmers University we will develop guidance for evaluation and reporting of statistical tests used in ecotoxicity studies. The manuscripts currently in preparation will be finalized and submitted for publication. We will also update the WikiPharma database (www.wikipharma.org).

Staff

In 2014, the following personnel have been directly involved in the project:
- Christina Rudén
- Marlene Ågerstrand

Publications

Published manuscripts

Molander L, Ågerstrand M, Beronius A, Hanberg A, Rudén C. 2014. "Science in Risk Assessment and Policy (SciRAP): An Online Resource for Evaluating and Reporting In Vivo (Eco) Toxicity Studies." Human and Ecological Risk Assessment 21 (3):753-762.

Ågerstrand M, Edvardsson L, Rudén C. 2013. Bad reporting or Bad Science? Systematic Data Evaluation as a Means to Improve the Use of Peer-Reviewed Studies in Risk Assessments of Chemicals. Accepted for publication in Human and Ecological Risk Assessment.

A Beronius, L Molander, C Rudén, Hanberg A. "Facilitating the use of non-standard in vivo studies in health risk assessment of chemicals – a proposal to improve evaluation criteria and reporting." Accepted for publication in Journal of Applied Toxicology.

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.

Manuscripts in preparation/submitted

Ågerstrand M, Kase R, Korkaric M, Moermond C. Towards more consistency and transparency in risk assessment. *Submitted.*

Ågerstrand M, Berg C, Björlenius B, Breitholtz M, Brunstrom B, Fick J, Gunnarsson L, Larsson DGJ, Sumpter PJ, Tysklind M, Rudén C. "Improving environmental risk assessment of human pharmaceuticals". *Submitted.*

Kase R, Moermond C, Korkaric M, Werner I, Ågerstrand M. "Comparison of the Klimisch and CRED methods to evaluate reliability and relevance of ecotoxicity studies". *Submitted.*

Moermond C, Kase R, Korkaric M, Ågerstrand M. "CRED - Criteria for Reporting and Evaluating ecotoxicity Data. An improved method for reliability and relevance evaluation". *Submitted.*

Ågerstrand M, Kase R, Korkaric M, Moermond C. "CRED - Criteria for Reporting and Evaluating ecotoxicity Data. Reporting recommendations". *Submitted.*

Kase R, Moermond C, Korkaric M, Werner I, Ågerstrand M. "Functionality and perception of the newly developed CRED method to evaluate reliability and relevance of ecotoxicity studies." *Manuscript.*

Ågerstrand M, Beronius A." Weight of Evidence in EU chemical regulations: Sometimes mentioned, rarely defined and never described." *Manuscript.*

Bergman Å, Ruden, C, Ågerstrand M, et al. "A framework for systematic review and evidence integration of endocrine disrupting chemicals." *Manuscript.*

Reviews and book chapters

Ågerstrand M, Breitholtz M and Rudén C. Reporting and evaluating ecotoxicity data for environmental risk assessment How can current practices be improved" in Analysis Removal Effects and Risk of Pharmaceuticals in the Water Cycle 2nd edition, Eds: Mira Petrovic, Sandra Pérez and Damià Barceló, Elsevier (expected >release 27 Nov 2013).

Publications associated projects

Bloch Hartmann N, Holten Lützhoft H-C, Ågerstrand M, Baun A. "Evaluation of nano-ecotoxicity studies for regulatory risk assessment of chemicals." Manuscript.

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

Hanna Netteberg, 2014. Evaluation of the reliability and relevance of toxicity studies of bisphenol A using the SciRAP-framework. Stockholm University, Stockholm, Sweden.

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

Conference presentations

Moermond C, Kase R, Korkavic M, Ågerstrand M. 2014. CRED - Criteria for Reporting and Evaluating ecotoxicity Data improve transparency and consistency. Platform presentation at SETAC North America in Vancouver, Canada.

Ågerstrand M, Kase R, Korkaric M, Moermond C. 2014. Criteria for Evaluating and Reporting Ecotoxicity data (CRED) - Report from a ring test. Platform presentation at SETAC Europe in Basel, Switzerland.

Sobek A, Ågerstrand, M. 2014. How effective is the Water Framework Directive in reducing levels of hazardous substances in the Baltic Sea? Poster presentation at SETAC Europe in Basel,

Switzerland. Rudén C. 2013. EDC - Policy development and regulatory needs. Presentation at the FEMREP conference in Uppsala.

Teaching - undergraduates and practioners

Ågerstrand was the course leader of the course "Strategies for Environmental Risk and Hazard Assessments" at Stockholm University. Ågerstrand have been teaching at the following courses: "Disturbed systems", "Introduction to environmental science" (Stockholm University); "Risks in technical systems" (Royal Institute of technology); "Environmental Toxicology" (Uppsala University). 2014.

Rudén has given lectures for undergraduates at Stockholm University, Karolinska Institutet and the Royal Institute of Technology. Ågerstrand has given lectures for undergraduates at Stockholm University and the Royal Institute of Technology. Rudén gave a lecture about MistraPharma and pharmaceuticals in the environment to "Senioruiversitetet". 2013.

Ågerstrand has developed and are the course leader of the course "Strategies for Environmental Risk and Hazard Assessments" (MI8009, 15 ECTS) at ITM, Stockholm University. She has also been the examiner of a candidate thesis at ITM. 2013.

Stockholm University

Project leader: Magnus Breitholtz

Summary of completed research 2014

In the second phase of the program, this project primarily focuses on evaluating new treatment technologies for removal of APIs from municipal wastewater. With consideration to removal efficiency, ecotoxicity and costs our previous work, both before MistraPharma started and in the first phase, showed that ozone and activated carbon are the two most promising treatment options to proceed with. However, although ozone effectively removes drugs, there are concerns that the strong oxidation causes formation of transformation products that are equally or more toxic than the mother compounds. In fact, there are indications that the oxidative process may create cascades of free radicals, which potentially need to be taken care of before releasing wastewater to recipients. In 2014, we therefore continued studying ozone treatment in combination with activated carbon/sand filtration as final treatment steps. Extending the work from the end of 2013 we have in 2014 tested yet a number of different wastewaters from the mobile MistraPharma pilot plant, both from its visit in Käppala and Uppsala. Wastewaters from the third and last visit in Västerås will be tested in 2015.

To complement our ecotoxicity tests focussing on adverse endpoints (i.e., mortality, development, reproduction and growth inhibition), we invested a lot of time and efforts in 2013 to develop and adopt a battery of sensitive and rapid biochemical (antioxidant enzymes and oxidized products) and molecular biomarkers (expression of genes related to antioxidant production) of oxidative imbalance. Our focus on oxidative imbalance is not only related to the fact that oxidation processes may generate aggressive transformation products, but also because evidence is accumulating that oxidative stress is a common denominator underlying many diseases and environmental insults. It is also becoming clear that a variety of contaminants, including drugs, may cause their deleterious effects, directly or indirectly, via reactive oxygen species (ROS) generation. The metabolic costs for antioxidant defences and stress recovery link oxidative stress to higher-level responses, as any increase in investment in the antioxidant system can only come at a cost to investment elsewhere. We are keen on improving our understanding of the relationship between markers of oxidative imbalance

and effects on growth and reproduction in both algae and crustaceans. This is crucial to make sure that the biomarkers are useful as screening tools but will also help us understand underlying mechanisms of toxic effects. One of the PhD students in the project, Sara Furuhagen, is working with oxidative stress and its relation to adverse effects, primarily in crustaceans but also in algae. In 2014, she published two scientific articles on the topic and will defend her doctorate thesis in the fall of 2015. We have also two technicians (Karin Ek and Birgitta Liewenborg) working more or less full time to optimize different biomarker techniques for both algae and crustaceans, which have been applied on wastewaters from the pilot plant at Käppala and Uppsala.

So far, the effluent waters that we have tested at both Käppala and Uppsala have induced relatively weak effects using both the algal and crustacean tests applied. Still, our preliminary results from these evaluations shows that while activated carbon and sand filtration have little impact on the toxicity of the effluent water released from these treatment plants, treatment with ozonation reduces toxicity to both Daphnia and micro algae. The fact that we have been evaluating effluent waters with low toxicity obviously makes it difficult to study additional positive treatment effects by e.g. ozonation as well as study the potential negative effects due to formation of transformation products. In 2014, we therefore hired a German master student (Malte Posselt) to study the production of transformation products in more depth. In collaboration with the German Environmental Protection Agency, three APIs [i.e. clarithromycin (CL), 1H-benzotriazole (BZ), carbamazepine (CBZ)] were selected for this purpose. Effects on different test organisms according to OECD TGs: 201 (Desmodesmus subspicatus), 221 (Lemna minor) and 236 (Danio rerio) were studied. In parallel, we searched for TPs in ozonated samples using HPLC/Q-TOF. Removal efficiencies and test concentrations were measured by UHPLC MS/MS. Ozone treatment was carried out at the MistraPharma pilot plant situated at the Käppala municipal sewage treatment plant (STP). Either effluent water from the STP or tap water (TW) was spiked with defined concentrations of the chemicals, ozonated, aerated and finally sand filtered. We applied retention times (20 min) and ozone doses (0.6 g O3/g TOC) that were previously described as economically feasible for large-scale application in STPs. CL and CBZ were effectively removed by about 99 % and 100 %, respectively. BZ proved as more resistant and variable removal rates between 0.2 % and 63.2 % were achieved. 15 potential ozonation TPs were identified of which 10 were previously detected in laboratory scale ozonation experiments. Clarithromycin-N-oxide was identified as the major TP of CL. Our results suggest that laboratory data from ozonation studies can be used to predict removal rates under STP condi-

tions. EW and TW partially covered the toxicity of both CL and BZ towards D. subspicatus and L. minor, respectively. Determined threshold concentrations and EC values were similar to literature data. It should be taken into account that BZ-induced alterations of frond shape of L. minor plants were observed at concentrations (3.97 mg/L) below the determined LOEC level. Furthermore, evidence for CL-induced hormesis in Desmodesmus subspicatus was found. Algae growth was increased, starting at analytically confirmed concentrations as low as 0.3 µg/L. This was in the range of concentrations reported for surface waters. The TP mixtures of both substances induced no increased toxicity compared to the parent compounds. CL toxicity towards algae was fully eliminated and no hormesis effects were observed after ozonation. Follow-up sand filtration improved the positive net-effect of ozonation by reducing residual concentrations of parent compounds and the concentrations of most TPs. Ozonation with comparably low doses of ozone proved as effective for the removal of priority pollutants from effluent water. The formation of TPs was observed but we found no hints for an increased toxicity of these compounds towards sensitive organism groups.

Lastly, in 2014 Elin Lundström Belleza defended her doctorate thesis: Population modeling using harpacticoid copepods: Bridging the gap between individual-level effects and protection goals of environmental risk assessment. The thesis aims to bridge the gap between what is measured and what is intended for protection in environmental risk assessment. By using population models, individual-level effects can be integrated in mathematical models, and translated to the population level. Matrix models and individual based models were developed and applied to life-history data of crustaceans commonly used in environmental risk assessment activities. As a basis for the population models, individual-level processes were studied. In general, development was found to be more sensitive compared to reproduction in standard ecotoxicity tests measuring life-history traits. Individual-level effects were more or equally sensitive compared to population-level effects, and individual-level effects were translated to the population level to various degrees by population models of different complexities. More complex models showed stronger effects at the population level compared to the simpler models. Density dependence was shown to affect crustacean populations, and toxicant effects were stronger at higher population densities. The tools presented in the thesis can be used to assess the toxicity of environmental contaminants at the individual and population level, improve ERA, and thereby the basis for environmental management.

Plans for 2015

The plan for 2015 is to finalize our evaluations of different wastewater treatment technologies. Two to three scientific papers will be written during the year together with KTH and Umeå. Sara Furuhagen will submit her last paper and defend her doctorate thesis in the fall of 2015. The last article from Elin Lundström Bellezas thesis will also be submitted.

Staff

In 2014, the following personnel have been involved in the project:

- Magnus Breitholtz (Professor)
- Elena Gorokhova (Professor)
- Karin Ek (technichian)
- Birgitta Liewenborg (technichian)
- Sara Furuhagen (PhD student)
- Elin Lundström (PhD student)
- Malte Posselt (master student)
- Samuel Moeris (master student)
- Josef Koch (master student)

Publications

Published manuscripts

Furuhagen S, Fuchs A, Lundström Belleza E, Breitholtz M, Gorokhova E (2014) Are pharmaceuticals with evolutionary conserved molecular drug targets more potent to cause toxic effects in non-target organisms? PloS One 9(8). Pages: e105028.

Furuhagen S, Liewenborg B, Breitholtz M, Gorokhova E. (2014) Feeding activity and xenobiotics modulate oxidative status in Daphnia magna: implications for ecotoxicological testing. Environmental Science and Technology 48 (21), 12886–12892.

Lundström Belleza E, Brinkmann M, Preuss TG Breitholtz M. (2014) Population-level effects in Amphiascus tenuiremis: Contrasting matrix- and individual-based population models. Aquatic Toxicology 157, 207-214.

Eriksson Wiklund A-K, Adolfsson Erici M, Liewenborg B, Gorokhova E (2014) Sucralose induces biochemical responses in Daphnia magna. PLOS ONE 9(4): e92771

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

Gorokhova E, Rivetti C, Furuhagen S, Ek K, Edlund A, Breitholtz M. Microbiome-mediated effects of trimethoprim in Daphnia magna. *Submitted to Environmental Science and Technology.*

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.

Lundström Belleza E, Breitholtz M. Interactions in high- and low-density populations and increased sensitivity of reproductive endpoints in Nitocra spinipes. *To be submitted to Aquatic Toxicology.*

Reviews and book chapters

Breitholtz, M. Crustaceans. (2013) In: Endocrine Disrupters: Hazard Testing and Assessment Methods (Editor: Mathiessen, P.), John Wiley & Sons, Inc, NJ, USA. ISBN: 978-0-470-93209-4.

Ågerstrand, M., Breitholtz, M., Rudén, M. (2013) Reporting and Evaluating Ecotoxicity Data for Environmental Risk Assessment: How Can Current Practices Be Improved? In: Analysis, Removal, Effects and Risk of Pharmaceuticals in the Water Cycle — Occurrence and Transformation in the Environment (Editors: Mira Petrovic, Damia Barcelo and Sandra Pérez. Comprehensive Analytical Chemistry 62, 685–704.

Doctoral Thesis

Elin Lundström Belleza (2014) Population modeling using harpacticoid copepods: Bridging the gap between individual-level effects and protection goals of environmental risk assessment. Doctoral thesis in Applied Environmental Science at Stockholm University. ISBN: 978-91-7447-894-5.

Furuhagen, S (2013) Integrative approaches in ecotoxicological testing: Implications for biomarker development and application. Licentiate thesis in applied environmental science.

Master Thesis

Malte Posselt (45 ECTH); work on transformation products from APIs extended over 2014-2015.

Josef Koch (45 ECTH); work in population models extended over 2014-2015.

Samuel Moeris (45 ECTS); work on mixtures of APIs extended over 2013-2014.

Conference contributions

Furuhagen S, Reutgård M, Breitholtz M, Gorokhova E. (2014) Biomarkers of oxidative stress as indicators of reproductive effects in the benthic amphipod Monoporeia affinis. Platform presentation at SETAC Europe, 24th Annual Meeting, Basel, Switzerland, 11-15 May 2014.

Lundström Belleza E, Breitholtz M (2014) Population growth rate in low- and high density populations. Platform presentation at SETAC Europe, 24th Annual Meeting, Basel, Switzerland, 11-15 May 2014.

Furuhagen S, Liewenborg B, Breitholtz M, Gorokhova E (2013) Feeding activity and oxidative stress in Daphnia magna. Platform presentation at the SETAC Europe 23rd Annual Meeting in Glasgow, Scotland.

Bui T, Lundström E, Breitholtz M, Schaeffer A, Preuss T (2013) Using individual based modeling to quantify the importance of sub-lethal effects on population level - a case study for Nitocra spinipes. Platform presentation at the SETAC Europe 23rd Annual Meeting in Glasgow, Scotland.

Breitholtz M (2013) Våtmarkers reningseffekt på läkemedel. Invited speaker at international conference ”20 år med spillvattenvåtmarker i Sverige”, Arrangörer: Nynäshamns kommun, WRS Uppsala AB och Oxelö Energi i samarbete med Stefan Weisner, Högskolan i Halmstad och Karin Tonderski, IFM Biologi, Linköpings Universitet samt EviEM, Mistras råd för evidensbaserad miljövård, 22-23 of May, Nynäshamn, Sweden.

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.

Umeå University

Project leader: Mats Tysklind and Jerker Fick

Summary of completed research 2014

Evaluation of High Risk APIs

Identification and evaluation API's of high concern has continued during 2014 and this work is still based on the fish plasma model, i.e. calculations based on the human therapeutic plasma concentration. We have started to focus on processes and have e.g. studied the tissue distribution of antidepressants in muscle, brain, liver, and plasma in fish (Grabikova et al 2014). We have also studied the effect of trophic interactions and subsequent bioaccumulation (Brodin et al 2014). Our study focusing on levels of pharmaceuticals found in fish plasma from caught wild fresh water fish in the UK, the Czech Republic and Germany is near completion and is planned to be submitted mid-2015. Additional studies have been performed investigating bioconcentration, one focusing on invertebrate aquatic species (Jonsson et al 2014) and one focusing on bioaccumulation (Heynen et al 2015, submitted manuscript).

As a measurement of persistency of API's, a study focusing on photo degradation experiments was done 2012 and a revised manuscript has been resubmitted (Golokova et al, submitted manuscript). In addition a more detailed study was performed 2014, focusing on degradation product formed from 10 selected pharmaceuticals, all experiments have been completed and a manuscript will be written in 2015. Studies have also focused on multivariate evaluation of the correlation between rate of photo degradation and physicchemical properties. Our study of pharmaceuticals in marine environment with focus on the Baltic Sea has been expanded and now includes samples from the Skagerrak, Barents Sea and the Greenland Sea. All sampling and analysis have been completed and a manuscript will be submitted during spring 2015.

Antibiotic Resistance

Field studies of antibiotic residues in effluent and sludge from operating, full-scale Swedish sewage treatment plants have been performed. This study has now been expanded and also includes biocides and analytical methods have been developed for these within the research project Interact. Our

field study of antibiotics and antibiotic resistance genes in River Stångån in Linköping have been published (Berglund et al). This study focused on the local sewage treatment plant as a source of antibiotics and antibiotic resistance genes and concluded that the levels of antibiotic resistance genes in River Stångån was correlated to the discharge of genes and not antibiotics.

Removal of prioritized APIs in Waste Water Treatment

Our major topic during 2014 has been to support KTH to optimize and validate the ozone- and/or carbon-based tertiary treatment at three different sewage treatment plants. This work will result in several manuscripts.

Analytical determinations

The development of an improved screening method for determination of +100 APIs was recently published (Lindberg et al. 2014). A sensitive and extended analytical method for progestines, estrogenes and androgenes (LOQ at 0.01 ng/L) has been developed and two manuscripts are being written. The sample throughput, as support for the whole MistraPharma program, has been high and a total of about 1800 samples have been analyzed during 2014, excluding lab and field blanks, standards and QA / QC runs.

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. We also contributed to the joint paper on improving environmental risk assessment of human pharmaceuticals (Ågerstrand et al 2015, submitted manuscript). We also published a paper which emphasis the importance of selecting relevant end-points when evaluating the ecotoxicological and ecological effects of pharmaceuticals in the environment (Klaminder et al 2014).

Plans for 2015

We will complete our on-going studies and primarily focus on supporting the final studies, with special emphasis on supporting KTH to optimize and validate the ozone- and/or carbon-based tertiary treatment.

Staff

Jerker Fick, Richard Lindberg, Marcus Östman, Annelie Lagesson, Jana Späth, Patrik Andersson, Alexandra Rybakka, Hanna Söderström and Mats Tysklind.

Publications

Published manuscripts

Almroth BC, Gunnarsson LM, Cuklev F, Fick J, Kristiansson E, Larsson DGJ. 2015 Waterborne beclomethasone diproprionate affects fish while its metabolite beclomethasone is not taken up. Science of the Total Environment 511, 37-46.

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Doctoral thesis

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Conference contributions

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Teaching - undergraduates and practioners

Lectures on fate and effects of pharmaceuticals have been given on several courses at the Department of Chemistry.

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 2014

Between January 2014 and now, the Gothenburg team has published 5 original articles and 8 book chapters, reviews and opinion papers with support from Mistra. In addition, we have presented a large number of papers at conferences. We have preliminary results for more than a handful papers in preparation. Here, we briefly highlight a few on our recent studies.

Larsson contributed to two reviews in the special issue on pharmaceuticals in the environment in Philosophical Transactions of the Royal Society B. In one review (Kookana et al) an attempt was made to place a global perspective on the emissions of pharmaceuticals, taking into account region-specific data and conditions. China and India stood out as two countries where risks are particularly high. The other review, with Larsson as sole author, compiled and analysed the available literature on discharges of active pharmaceutical ingredients from manufacturing as well as effects associated with such discharges. In addition to pointing out the much higher concentrations, and thus much larger risks, associated with manufacturing discharges (compared with emissions from usage), he concluded that large emissions from manufacturing are widespread and concerns everyone, particularly if antibiotics can promote resistance development. Risk management also differ from discharges from excretion in terms of accountability, incentives creation, legal opportunities, substitution possibilities and costs.

Bengtsson-Palme et al also published a paper in Frontiers in Microbiology describing the environmental resistome of an antibiotic-contaminated lake in India. In addition to unprecedented numbers of resistance genes for basically all classes of antibiotics, the results also suggested an exceptional adaptation of the bacteria to exchange genes with each other. Possibly, this is a strategy for the bacteria to survive such extreme environments, and it clearly enhances the risks for resistance gene transfer to human pathogens.

We also published two papers on pharmaceuticals in fish, one on the glucocorticoid beclomethasone and how it bioconcentrates and affect the physiology of exposed trout (Carney-Almroth et al), and one paper on the accumulation of antidepressant in different tissues of the fish (Grabicova et al). In the latter, we concluded that measuring the drug in the actual target organ (here the brain) may be more informative for risk assessments than attempting to measure it in the blood plasma of the fish.

We were also pleased that Nature Reviews Microbiology, the highest cited journal in the field of microbiology, recently decided to publish a short commentary article by us (Bengtsson-Palme and Larsson). Publishing in high-impact journals with a broad scope can be important in order to spread awareness about pharmaceuticals in the environment to a wider scientific audience.

Plans for 2015

We anticipate to dedicate some effort to handle review-comments on papers already submitted (see below). We hope to be able to submit a manuscript on the effects of water-born diclofenac on sticklebacks (in collaboration with SLU). As part of this collaboration, we also hope results will be completed for the exposure study we performed at Uppsala sewage treatment plant during 2014. We hope that Lina Gunnarsson, who has received a position at Exeter university in the UK, will find the time to complete the manuscripts for two papers on bioconcentration and effects of NSAIDs in fish, as well as preparing the manuscript on the relation between the origin of pharmaceuticals, environmental control in the region of production and the final sales price. We also plan to finalize a paper on antibiotic resistance genes in different steps of the sewage treatment process at three sewage treatment plants. We hope the Umeå-node will find the time to draft the collaborative papers on bioconcentration in wild freshwater and saltwater fish, respectively (all analyses are completed). The last practical experiment we expect to perform within the programme is one where we will use sterile-filtered effluent to assess its ability to select for antibiotic-resistant bacteria, and one experiment using the mobile experimental container to assess the effects of, primarily, ozonation on resistant bacteria and resistance genes.

Staff

Lina Gunnarsson, who has been working within the MistraPharma programme already from the idea-support stage (2004 and onwards) in the Larsson lab, has received a researcher position at Exeter university, UK, in Prof Charles Tyler´s laboratory. There, she will primarily work within the

IMI project "iPiE: Intelligent led assessment of pharmaceuticals in the environment". Anna Johnning successfully defended her thesis in April 2014 and now works both as a postdoctoral fellow with Erik Kristiansson at Chalmers, and in Kristiansson´s company "1928diagnostics", developing solutions to rapidly identify the resistance profile of bacteria causing infections through DNA sequencing data. Johan-Bengtsson Palme plan to defend his PhD thesis in early 2016 (extended due to parental leave). Drs Carl-Fredrik Flach, Sara Lundström, Jekaterina Jutkina, Carolin Rutgersson and Nachiket Marathe in the Larsson laboratory all work part-time within MistraParma, as does Larsson. Our technician Birgitta Weidegård retired during 2014. At the Chalmers node, docent Erik Kristiansson and PhD student Fredrik Boulund both work part-time within MistraPharma.

Publications

Published manuscripts

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Johnning A, Kristiansson E, Fick J, Weijdegård B, Larsson DGJ. Resistance mutations in gyrA and parC are common in bacterial communities of both fluoroquinolone-polluted and uncontaminated environments. *Submitted.*

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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.

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Publications associated projects

Pal C, Bengtsson-Palme J, Rensing C, Kristiansson E, Larsson DGJ. (2014) BacMet: antibacterial biocide and metal resistance genes database. Nucleic Acids Research, 42, D737-D743. doi: 10.1093/nar/gkt1252.

Asker N, Kristiansson E, Albertsson E, Larsson DGJ, Förlin L. 2013. Hepatic transcriptome profiling indicates differential mRNA expression of apoptosis and immune related genes in eelpout (Zoarces viviparus) caught at Göteborg harbor, Sweden. Aquatic Toxicology. 130–131:58-67. doi: 10.1016/j.aquatox.2012.12.017.

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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

Carolin Rutgersson. Environmental pollution from pharmaceutical manufacturing - effects on vertebrates and bacterial communities. PhD thesis. 13 Sept 2013. University of Gothenburg. https://gupea.ub.gu.se/handle/2077/32956.

Anna Johnning. 2014. Fluroquinolone resistance in the environment and the human gut – analysis of bacterial DNA sequences to explore the underlying genetic mechanisms. PhD thesis, defended on the 24th of April, Sahlgrenska academy, University of Gothenburg. ISBN 978-91-628-8993-7.

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

Half-time controls/Licenciate thesis

Johan Bengtsson-Palme. Using Metagenomics to Investigate Effects of Pharmaceutical Pollution on the Environmental Resistome. University of Gotheburg. June 13 2013.

Fredrik Boulund. Analysis of large-scale metagenomic data. Chalmers University of Technology. October 18, 2013. http://publications.lib.chalmers.se/records/fulltext/183892/183892.pdf

Teaching - undergraduates and practioners

During 2014 we have taught aspects on pharmaceuticals in the environment on several undergraduate educational programmes in Gothenburg, including for example the Medical Doctors Programme, two Pharmacy Programmes, the Odontology, Programme, Nursing Programmes and more.

Larsson, Gunnarsson and Svensson have taught "pharmaceuticals in the environment" between during 2013 and 2014 on several undergraduate educational programs in Gothenburg, including for example the Medical Doctors Programme, two Pharmacy-programmes, the Odonotologyprogramme, Nursing programmes and more. Teaching has extended to nurses and medical practitioners at the Nordic School for Public Health (NHV).

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).

Conference contributions

We have contributed with a large number of presentations at various conferences. Here is a selection:

Larsson gave a one-hour Keynote lecture at ECCMID (European Conference on Clinical Microbiology and Infectious Diseases) in Barcelona in May 2014. A Keynote at such a large conference (10,627 delegates from 117 countries) provided an exceptionally good opportunity to communicate the environmental dimensions of antibiotic resistance to the leading clinical microbiologist in Europe.

Larsson also gave an Invited lecture during the opening day of the yearly conference arranged by the Japanese Society of Bacteriology in Tokyo, 2014.

Nachiket Marathe presented the Larsson-group´s research at an conference in Lisbon, Portugal in February, 2015. (1st International Caparica Conference in Antibiotic Resistance)

Larsson also gave the introductionary talk at "Antibiotikaforum 2014" in Stockholm. The theme for the meeting was the role of the external environment in antibiotic resistance.

Uppsala University

Project leader: Ingvar Brandt

Summary of completed research 2014

The observations that the progestagenic anticontraceptive drug levonorgestrel is a potent developmental and reproductive toxicant in frogs and fish is a key finding in the MistraPharma Uppsala program. Based on these results the work during 2014 has to a large extent been focussed on characterizing ecotoxicological effects of various synthetic progestins and of the natural hormone progesterone in aquatic vertebrates. Studies on how individual antifungal azoles and mixtures thereof can inhibit CYP enzyme catalytic activity in fish have been completed. The efficacy of new STP treatment technologies (operated by KTH) has been evaluated by means of various biomarkers and chemical analysis, using treated effluent water from the Stockholm Käppala and Uppsala STPs.

Recent key findings in the frog project are that the three progestogens levonorgestrel (LNG), norethindrone (NET) and progesterone (P) inhibited oogenesis in frogs by interrupting the formation of vitellogenic oocytes at an environmentally relevant concentrations, i.e. 1-10 ng/L. The progestagens are of particular concern given their prevalence, the range of compounds and that several of them (LNG, NET and P) share the same target (oogenesis) at environmental exposure concentrations, indicating a risk for adverse effects on fertility in exposed wild amphibians. Another important finding is sex-dependent ontogenetic mRNA expression of anti Müllerian hormone (amh) and amh receptor 2 in frogs. Furthermore, we have successfully completed an amphibian life-cycle experiment to determine developmental reproductive toxicity of the anti-androgenic pharmaceutical flutamide and the pesticide linuron. The analysis of the data from this experiment is currently on-going.

In studies using the three-spined stickleback androgen model, the progestagen levonorgestrel has been demonstrated to exert strong androgenic effects following water-borne exposure at environmentally relevant concentrations. These effects are manifested both as kidney hypertrophy with excessive spiggin production in females, and as a reduced ability of male fish to resume spermatogenesis by leaving the reproductive cycle. The latter effect is particularly noteworthy and likely of great ecological relevance. A follow-up

study in zebrafish shows that LNG also gives rise to a male-biased sex ratio with almost nearly all-male populations at 10 ng/L and higher concentrations of LNG. The concomitant observation that the natural hormone progesterone did not affect the sex ratio supports the contention that the androgenic activity of progestins could be particularly important in fish. In addition, both LNG and progesterone were found to induce precocious puberty along with altered expressions of gonadotrophic hormone transcript levels in the pituitary of zebrafish. These studies are ongoing and will be completed by the end of 2015.

A new method to measure CYP enzyme inhibition by pharmaceuticals in dissected gill filament tips has been developed and applied to examine a range of azole fungicides regarding their ability to inhibit CYP1A activity in rainbow trout gills. In comparative studies using rainbow trout brain microsomes, the ability of these azoles to inhibit CYP19 (aromatase) has been examined. The results show that some of these azoles are potent inhibitors of these catalytic CYP activities, implying that they can inhibit a variety of physiological functions including sex hormone production. Mixtures of the azoles studied were also shown to inhibit these CYP activities in an additive fashion.

Studies to evaluate new STP treatment technologies have been carried out using effluent water from the Käppala and Uppsala sewage treatment plants. Both active carbon filtration and ozonation were found efficient to further reducing the response of certain selected biochemical and molecular biomarkers as well as the concentrations of a variety of human pharmaceutcals in the purified effluents at both sites.

Plans for 2015

Kristina Beijer is scheduled to defend her PhD thesis (CYP inhibition and STP technology evaluation) in early June, while Erica Jansson will defend her Licentiate thesis (effects of progestins in frogs) in April. Johan Svensson will be close to completion of his PhD thesis (effects of progestins in fish) at the end of the year. The reproductive and developmental toxicity studies of various progestins in frogs will be given continued focus in 2015, and the search for molecular markers predicting down-stream alteration in reproductive organ histological structure and physiological function will be completed. In the zebrafish study the histological evaluation to determine final sex ratio and gonadal maturity indices will be completed. Real-time qPCR analyses of genes regulating sexual differentiation and puberty will also be finished and published in mid-2015. A study comparing the in vivo androgenic potency of various progestins and other steroid hormone pharma-

ceuticals (totally six compounds) as well as a mixture group will be carried out, including endpoints for spiggin induction and vitellogenin inhibition in female sticklebacks. This work will require chemical analysis. The studies on CYP modulation by azoles and the evaluation of the new STP technologies will be completed during the first six months.

Staff

Project leader: Ingvar Brandt, Prof

Kristina Beijer, PhD student, fish project
Cecilia Berg, Associate professor, frog project
Björn Brunström, Prof, fish and STP project
Erika Jansson, PhD student, frog project
Moa Säfholm, PhD student, frog project
Johan Svensson, PhD student, fish project

Publications

Published manuscripts

Säfholm M, Jansson E, Fick J, Berg C. 2015. Mixture Effects of Levonorgestrel and Ethinylestradiol: Estrogenic Biomarkers and Hormone Receptor mRNA Expression during Sexual Programming. Aquatic Toxicology, in press.

Säfholm M, Ribbenstedt A, Fick J, Berg C. 2014. Risks of hormonally active pharmaceuticals to amphibians: A growing concern regarding progestagens. Philosophical Transactions of the Royal Society B 369:1656

Svensson, J., Fick, J., Brandt, I., Brunström, B. 2014. Environmental concentrations of an androgenic progestin disrupts the seasonal breeding cycle in male three-spined stickleback (Gasterosteus aculeatus). Aquatic Toxicology, 147:84-91.

Beijer K, Gao K, Jönsson M, Larsson DGJ, Brunström B, Brandt I. 2013. Diluted effluent from drug manufacturing affects cytochrome P450 1 regulation and function in fish. Chemosphere 90, 1149-57.

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.

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, 47(4):2043-2051.

Bergman A, Heindel JJ, Kasten T, Kidd KA, Jobling S, Neira M, Zoeller RT, Becher G, Bjerregaard P, Bornman R, Brandt I, Kortenkamp A, Muir D, Drisse MN, Ochieng R, Skakkebaek NE, Byléhn AS, Iguchi T, Toppari J, Woodruff TJ. 2013. The impact of endocrine disruption: a consensus statement on the state of the science. Environ Health Perspect. 2013;121(4):A104-6.

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.

Manuscripts in preparation/submitted

Ågerstrand M, Berg C, et al. 2015. Improving environmental risk assessment of human pharmaceuticals. *Submitted.*

Jansson E, Mattsson A, Goldstone J, Berg C. 2015. Sex dependent ontogenetic expression of anti Müllerian hormone (amh) and amh receptor 2 in Xenopus (Silurana) tropicalis. *Submitted.*

Säfholm M, Jansson E, Fick J, Berg C. 2015. Effects of levonorgestrel on reproductive organ development and mRNA expression of anti-Müllerian hormone (amh) and progesterone receptors (pgrs) in Xenopus (Silurana) tropicalis. *In preparation.*

Beijer K, Jönsson M, Shaik S, Behrens D, Brunström B, Brandt I. 2015. Azole fungicides inhibit CYP1(ethoxyresorufin deethylase; EROD) and CYP19A (aromatase) activity in rainbow trout (Oncorhynchus mykiss) in an additive fashion. *Manuscript.*

Beijer K et al. 2015. Reduction of contaminants in effluent water from municipal sewage treatment plants: Evaluation of activated carbon filtration and ozonation. *Manuscript.*

Reviews and book chapters

Berg C, Lundstedt-Enkel K, Olovsson M, Persson S (eds). 2013. Female Reproduction and Endocrine Disrupting Chemicals (FEMREP 2013). Proceedings from an international conference at Centre of Reproductive Biology, CRU report no 28, Uppsala, Sweden.

Publications associated projects

Zoeller RT Bergman Å, Becher R, Bjerregaard P, Bornman R, Brandt I et al. 2014. A path forward in the debate over health impacts of endocrine disrupting chemicals. Environmental Health, 14,118-

Doctoral Thesis

Moa Säfholm; Developmental and Reproductive Toxicity of Progestagens in the Xenopus (Silurana) tropicalis Test System. Completed in December 2013; defended 14 February 2014.

Kai Gao; Basal and Pollutant-induced Expression of CYP1A, 1B and 1C isoforms in Fish: Implications for Biomonitoring. Defended 28 May 2013.

Licentiate Thesis

Johan Svensson; Androgenic Effects of the Progestin Levonorgestrel in Three-spined Stickleback (Gasterosteus aculeatus). Defended March 2014.

Master Thesis

Ribbenstedt A. 2014. Exposure to progesterone or norethindrone disrupts oogenesis in the West-african clawed frog (Xenopus tropicalis). MSc report no. 14:032, Master Programme in Environmental Toxicology, Uppsala University.

Carlsson Y. 2014. Developmental reproductive effects of the anti-androgenic pesticide linuron on the frog Xenopus tropicalis. MSc report no. 14:049, Master Programme in Environmental Toxicology, Uppsala University.

Nick Jindrisek, 2013. Exponeringsförsök på yngel av Xenopus tropicalis med miljörelevanta progesteronkoncentrationer. Master programme in pharmacy. Dept of Pharmaceutical biosciences/Dept of Environmental Toxicology, Uppsala University.

Victor Tsiamis. Biomarker response in rainbow trout (Onchorynchus mykiss) exposed to additionally treated sewage treatment plant effluent. ISSN 1400-4178, Kph Uppsala 2013.

Conference presentations

Säfholm M, Jansson E, Fick J, Berg C. Mixture Effects of Levonorgestrel and Ethinylestradiol on Hormone Sensitive Biomarkers during Sexual Programming in Xenopus (Silurana) tropicalis. ISAREN 2014, The 8th International Symposium on Amphibian and Reptilian Endocrinology and Neurobiology. Okazaki, Japan, Nov. 7 - 9, 2014

Orton F, Erika Janson E, Carlsson Y, Eriksson A, Säfholm M, Fick J, Tyler CR, Berg C. Features of reproductive biology in X. tropicalis. ISAREN 2014, The 8th International Symposium on Amphibian and Reptilian Endocrinology and Neurobiology. Nov. 7 - 9, 2014, Okazaki, Japan,

Orton F, Erika Janson E, Säfholm M, Carlsson Y, Eriksson A, Fick J, Tyler CR, Berg C. The antiandrogens linuron and flutamide alter molecular and behavioural endpoints in male clawed frogs (Xenopus tropicalis). Gordon Research Conferences: Environmental Endocrine Disruptors, May 11-16, 2014, Lucca (Barga), Italy.

Berg C. Müllerian duct dysgenesis - a common cause for female reproductive disorders? Gordon Research Conferences: Environmental Endocrine Disruptors, May 11-16, 2014, Lucca (Barga), Italy.

Svensson J. 2014. Developmental exposure to progestins cause male bias and precocious puberty in zebrafish (Danio rerio). SETAC Europe Annual Meeting, May 2015.

Berg C. Risks of progestagens to amphibians. European Federation for Pharmaceutical Sciences (EUFEPS) conference: Human Pharmaceuticals in the Environment - Challenges in research and the need for societal action, June, 2013. Invited speaker.

Berg C. Müllerian duct differentiation – a sensitive target for endocrine disrupters in amphibians. SETAC (International Society of environmental toxicology and chemistry), Glasgow, UK, 2013. Platform presentation.

Berg C & Säfholm M. Reproductive toxicity of progestogens – norethindrone and progesterone inhibit vitellogenesis. SETAC, Glasgow, UK, 2013. Poster.

Berg C, Säfholm, M, Jansson, E, Fick J, Brandt I. Effects of Progestin and Estrogen Mixtures: A partial life cycle study on sex differentiation. SETAC, Glasgow, UK, 2013. Poster.

Berg C. Oral presentation on reproductive effects of APIs in amphibians at the conference Female Reproduction and Endocrine Disrupting Chemicals (FEMREP), October 2013. Platform presentation.

Jansson E, Mattsson A, Goldstone J, Olsson J, Berg C. The frog model for developmental reproductive toxicity: Anti-Müllerian hormone mRNA expression during sex differentiation in Xenopus tropicalis. The conference Female Reproduction and Endocrine Disrupting chemicals (FEMEREP) 2013. Poster.

Svensson, J., Brandt, I., Brunström, B. The progestin levonorgestrel is a potent androgen in the three-spined stickleback (Gasterosteus aculeatus). Platform presentation at the Pollutant Responses in Marine Organisms (PRIMO) 17 Congress, Faro, Portugal, 5-8 May 2013. Johan Svensson was awarded price for best platform presentation.

Svensson, J., Brandt, I., Brunström, B. The progestin levonorgestrel is a potent androgen in the three-spined stickleback (Gasterosteus aculeatus). Poster presentation at the workshop "Endocrine Disrupting Chemicals and Female Reproduction (FEMREP)", Evolutionary Biology Centre, Uppsala, Sweden, 5-6 November 2013.

Säfholm M & Berg C. Environmental concentrations of norethindrone and progesterone inhibit egg development in amphibians. The conference Female Reproduction and Endocrine Disrupting Chemicals (FEMREP) 2013. Poster.

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.

Teaching - undergraduates and practioners

Åberg, D. 2014. Progestins and the progesterone receptors – Mediating developmental toxicity in the female urogenital tract. Research internship report, 15 credits. Dept of Environmental Toxicology, Uppsala University.

Royal Institute of Technology (KTH)

Project leaders: Gen Larsson
Assistant project leader: Berndt Björlenius

Summary of completed research 2014

In the beginning of 2014 the 12 lines in the mobile lab were started up again at Käppala WWTP. The lines were in continuous operation most of the time. From time to time, flow proportional pumping was undertaken to study the resulting behaviour in the removal processes. In addition to continuous operation, factorial experiments with e.g. dose, temperature and pH were performed.

After the first four months of operation, sampling and preliminary evaluation, the pilot plant was moved to Kungsängsverket WWTP in Uppsala. The disassembling and assembling of the plant were relatively efficient, in less than two weeks, the plant was up and running in Uppsala. The first relocation showed that much time was used packing smaller and larger objects on several pallets. To facilitate the packing and storage a storage container was purchased. In the same container a small office space was furnished.

One reason of moving the pilot plant was to study the effect of wastewater composition and type of treatment on the treatability of pharmaceutical residues. The most obvious result, showing just after a few days, was that without a final sand filter at the treatment plant, the up time for granular activated carbon filters (GAC) was limited. To prolong the uptime for the GAC-filters a pre treatment in form of sand filters was built ahead of the pilot plant.

In Uppsala additional factorial experiments were undertaken in parallel with continuous operation of the 12 lines. In the end of September the pilot plant was disassembled again and moved to WWTP in Västerås.

From mid October continuous operation and factorial experiments were commenced in Västerås. The same observation was made here about the necessity of pre treatment to prolong the operation of GAC. In the beginning of December the operation was shut down. 15 december the pilot plant was moved back to Käppala WWTP. There complementary tests will be done on pre treatment, control of ozone addition, GAC and powdered activated carbon (PAC).

During the autumn we designed Sweden´s first full scale ozonation treatment step to remove micropollutants. The ozonation step is now under construction in Knivsta as the final treatment step in the existing mechanical, biological and chemical wastewater treatment plant (WWTP). Knivsta WWTP was chosen due to an reasonable size, 10 000 inhabitants and the small receiving watercourse. The Swedish Agency for Marine and Water Management, SwAM, funds a major part of the project in Knivsta. During 2014 a mobile lab container for exposure tests in 12 fish tanks was designed and constructed.

A new sampling campaign of sea water was planned by KTH and performed by the crew on the ship "The Brig Tre Kronor af Stockholm", operated by the "Initiative Sustainable Seas". Modelling of the fate and flow of a handful substances in the Baltic Sea catchment areawas done in the end of the year and the results will be published in 2015. The sampling and modelling help to understand the fate of APIs in the effluent from existing wastewater treatment plants, later transported or processed in the receiving water, can help us to select the APIs that have to be removed in the wastewater treatment plants, instead of being accumulated in the environment.

One major exposure on rainbow trout was performed at Kungsängsverket in Uppsala during one week in the beginning of September. Three extensively treated effluent streams (sand filtrated, ozonated and granular activated carbon filtered) were compared with the negative control; tap water and the positive control; effluent from the treatment plant. The exposure was done in collaboration with Uppsala University. Samples for bio tests to be analysed at ITM, on e.g. Daphnia Magna were also collected during the exposure.

Plans for 2015

In 2015 complementary pilot tests will be done on pre treatment, control of ozone addition, GAC and powdered activated carbon (PAC) at Käppala WWTP. The full scale ozonation step at Knivsta WWTP will be completed, commissioned and operated for six months. The mobile lab with the fish tanks will be installed and adapted to the ozonation plant and watercourse.

Focus will however be on the evaluation of pilot and full scale tests. In total six-seven scientific papers have to be submitted in cooperation with other partners in MistraPharma.

Staff

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´s role.

Victor Kårelid is PhD-student and dedicates his research to the pilot lines with powdered activated carbon (PAC).

Anna Hjalmars ended her employment as process operator in beginning of March 2015; New operator is Pia Trygg. She purchases parts to the pilot plants and assists the building activities in the mobile lab. She has the majority of the daily operation in her hands.

Publications

Manuscripts in preparation/submitted

"Ozonation of retentate from NF and RO applied for removal of APIs from municipal wastewater" Björlenius B, Flyborg L, Larsson G.

"Evaluation of GAC and PAC in a mobile pilot plant at three wastewater treatment plants" Berndt Björlenius, Victor Kårelid, Gen Larsson

"A PLS model for predicting rejection of trace organic compounds by nanofiltration using treated wastewater as feed"; Lena Flyborg, Berndt Björlenius, Magnus Ullner and Kenneth M. Persson.

Master Thesis

Louise Jansson, KTH - "Removal of pharmaceuticals in wastewater".

Teaching - undergraduates and practioners

KTH Industrial Biotechnology, Course for 24 undergraduate students; six lectures and 36 h lab in Käppala WWTP. Three persons from the project planned and assisted the lab. Stockholm University, Lecture "End of pipe solutions – Waste water treatment" in the course; Strategies for Environmental Risk and Hazard Assessments. 2014.

KTH Industrial Biotechnology, Course for 24 undergraduate students; six lectures and 36 h lab in Käppala WWTP. Four persons from the project planned and assisted the lab. Berndt gave the lectures. 2013.

Conference contributions

Swedish Agency for Marine and Water Management, SwAM -"Läkemedel i vattnet – reningsteknik", Berndt Björlenius, Conference in Gothenburg, Maj 22.

Elmia Water and Wastewater conference program; "Reningsmetoder och ny teknik – hur kan vi få fram kostnadseffektiv teknik som fungerar i praktiken? Conference in Jönköping, October 2,

Uppsala, 27th of June 2013; EUFEPS Human Pharmaceuticals in the Environment – Challenges in research and the need for societal action - "Can the problem be solved at the end of the pipe?" Berndt Björlenius

Xiamen, 5th-8th of December 2013; International Workshop on the Environmental Dimension of Antibiotic Resistance – "Removal of Antibiotic Substances in Existing and Extended STPs - full scale and pilot tests in Stockholm, Sweden" Berndt Björlenius

Brunel University

Project leaders: John Sumpter

Summary of completed research 2014

A multicomponent mixture study using five steroidal pharmaceuticals was tested in a well-established fish reproduction assay. This work was completed in October 2014. The purpose of the study was to assess the potential for combination effects on an ecologically relevant end point – reproduction. Five independent in vivo studies were performed prior to the mixture study to assess activity of each compound individually. A mixture study was subsequently designed based on mathematical modelling of single compound data. Using regression modelling and the mixture prediction models Concentration Addition (CA) and Independent Action (IA), the multicomponent mixture experiment was designed based on a fixed equipotent mixture ratio of the five compounds. The experiment was carried out during the months of August-October 2014.

Statistical analysis has been performed on many of the end points, i.e. egg production, secondary sexual characteristics etc., and is ongoing. This work is in collaboration with Martin Scholze.

Chemical analysis of experimental water samples is ongoing. Two chemicals, Trenbolone and EE2 have been analysed at Brunel University using enzyme-linked immunosorbent assays (ELISA's), and a further compound (Levonorgestrel) will be tested using a Radioimmunoassay (RIA) during early 2015.

Further to this, water samples were sent to Umea University in March 2015 for analytical analysis of all five compounds.

Plans for 2015

During the first part of 2015, we will continue with the analysis of water samples from the mixture experiment undertaken in 2014. Tara will use a RIA to measure Levonorgestrel in the water samples.

We then hope to compare results of measured concentrations from the three chemicals analysed at Brunel to results produced using analytical techniques at Umea University.

Tara will use histological techniques to analyse gonad tissue from the aforementioned study to investigate pathological changes to the gonads as a result of exposure to the pharmaceutical mixture.

During the summer of 2015, a further in vivo experiment is planned, of which the primary aim is to expose fish to the same mixture of chemicals at the same concentrations under the same conditions, in order to collect pituitary tissue for analysis of Follicle Stimulating Hormone (FSH) and Luteinising Hormone (LH) transcripts. This will strengthen the reproductive results from the previous experiment, and add an additional level to the analysis.

Additionally, work on a manuscript for publication will continue during 2015. Tara will also begin working on her thesis towards the end of 2015, for planned submission in 2016.

Staff

Professor John Sumpter (Project Lead at Brunel)

Ms. Tara Thrupp (PhD Student)

Dr. Tamsin Runnalls (Post-doctoral fellow funded by another organisation)

Mr. Martin Scholze (Biostatistician)

Professor Andreas Kortenkamp (second supervisor of Tara Thrupp)

Publications

Manuscripts in preparation/submitted

Manuscript in preparation at Brunel: Reproductive and endocrine effects of mixtures of steroidal pharmaceuticals with diverse mechanisms of action in a fish reproduction assay.

Professor Sumpter contributed to the paper that Marlene Agerstrand wrote on how the EMA guidelines on the environmental risk assessment of pharmaceuticals can be improved. This paper has been submitted for publication.

Publications associated projects

Sumpter,J.P. and Margiotta-Casaluci,L. 2014. Are some invertebrates exquisitely sensitive to the human pharmaceutical fluoxetine? Aquatic Toxicology 146, 259-260.
Sumpter,J.P., Donnachie,R.L. and Johnson,A.C. 2014. The apparently very variable potency of the anti-depressant fluoxetine. Aquatic Toxicology 151, 57-60.

Arnold,K.E., Brown,A.R., Ankley,G.T. and Sumpter,J.P. 2014. Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems. Phil. Trans. R. Soc. B. 369, 20130569.

Johnson,A.C. and Sumpter,J.P. 2014. Putting pharmaceuticals into the wider context of challenges to fish populations in rivers. Phil. Trans. R. Soc. B. 369. 20130581.

Margiotta-Casaluci,L., Owen,S.F., Cumming,R.I., de Polo,A., Winter,M.J. Panter,G.H., Rand-Weaver,M. and Sumpter,J.P. 2014. Quantitative cross-species extrapolation between humans and fish: the case of the anti-depressant fluoxetine. PLOS One 9, e110467.

Teaching - undergraduates and practioners

The issue of pharmaceuticals in the environment, and their effects on wildlife, are covered in some lectures Professor Sumpter gives to Masters students at his university.

Conference contributions

Abstract accepted for platform presentation at SETAC Europe in Barcelona (May 2015). Tara Thrupp to present on the effects of mixtures of steroidal pharmaceuticals in a fish reproduction assay.

Graham Harris (PhD Student-funded by another project) will present a poster at SETAC Europe in Barcelona on mixture effects of cytotoxic pharmaceuticals in bioassay model species.

Dr Tamsin Runnalls presented a poster at SETAC North America in Vancouver (November 2014) entitled 'Single chemicals and binary mixtures: Effects of synthetic steroids on reproduction in the Fathead Minnow'.

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:

The reference group

MistraPharma have a dedicated and knowledgeable reference group of 21 different representatives from our stakeholders.

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

April 2

This was a telephone meeting where the communication project presented the work within the working group for "Pharmaceuticals and Environment 2016-2020, after the end of MistraPharma program". The proposal was discussed and the members gave suggestions of adjustments and complements. All the members of the reference group gave a short view of what interesting activities that is happening within respective organisation..

October 28

On requests from the project leaders parts of this year's reference group meeting was incorporated in the program's regular agenda for the program meeting. Project leaders unveiled the latest news from their projects and what activities that are planned for 2015. The reference group members had the opportunity to ask questions and discuss specific issues relating to the research. Thereafter the members of the reference group gave a brief sum-

mary of the main activities going on in their respective organisations. The separate reference group meeting aimed to discuss the reference group's needs and desires of communication activities during next year. To conclude the program meeting a brainstorming session was held together with the researchers and the board with the aim to come up with ideas of activities to sum up the eight years of the MistraPharma program. The meeting ended with a joint dinner with the researchers and the program's board of directors.

Information

Newsletter

During the year one newsletter have been published in English, in May. The newsletter 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 and seminars

Almedalen

MistraPharma arranged five exciting seminars during Almedalen week in the beginning of july together with the Sustainable Seas Initiative/Briggen Tre Kronor, the Chemicals Agency, the Medical Products Agency, Mistra, Trossa, the Baltic Sea Centre and the department of applied environmental science (ITM) at Stockholm University. The theme was chemicals (including pharmaceuticals) in our homes; in the kitchen, in the bathroom and in the childrens' room. How does our consumption affect the Baltic Sea and our health? Can we make more informed choices? Scientists, politicians, representatives from industry and government agencies discussed these questions and gave their recommendations. Both researchers and Board members from MistraPharma participated in the panels. The days were wrapped up by a debate, led by Folke Rydén,with representatives from all political parties (youth sections) about "The Baltic Sea - we care!" and tomorrow's environmental policies".

Exhibition together with Sustainable Seas Initiative

Together with Stockholm university MistraPharma communication project helped the the Sustainable Seas Initiative to develop their environmental exhibition "En etta med kök" which was inaugurated by Environment Minister Lena Ek Almedalen June 30, 2014. The theme of the environmental

exhibition is plastic, chemicals and pharmaceuticals in our home environment. The exhibition will tour in Sweden for two years and appears in about 20 cities.

MistraPharma after 2015

During the spring the Board adopted the proposal from the committee on how pharmaceuticals and the environment can go on 2016-2020. The committee has been led by the communication project.

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.

Website

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.

Other communication activities - MistraPharma researchers

Umeå University

Jerker Fick, Hållbar läkemedelsanvändning: nu och i framtiden, Landstingens nätverk för läkemedel och miljö tillsammans med Stockholms läns landsting, 2014-05-08.

Mats Tysklind – ”Havet – är det bara vatten du ser?” Föreläsning för allmänheten om läkemedel och andra miljögifter i våra hav. Ekenäs, Kosterhavets nationalpark, 2014-08-04, Västerhavsveckan 2014.

Jerker Fick, Östersjön–en naturlig skönhet? Hur påverkar läkemedel, kosmetika och hygienartiklar vår vattenmiljö? Almedalsveckan, 2014-07-01.

Jerker Fick, Medverkade I workshopen, “PIE EU Strategy Workshop”, Brussels, Belgien, 2014-09-11, 2014

Jerker Fick, HAV 2014 Havsmiljöseminarium, Örnsköldsvik, 2014-09-22

Jerker Fick, Norrländska regionala läkemedelsrådet, 2014-10-07.

Jerker Fick, Swetox Workshop, 2014-12-16.

University of Gothenburg

A selection of stakeholder-interactions are indicated below: Larsson gave two talks at the Swedish veterinary congress in Uppsala in 2014. He also gave a presentation at meeting in Stockholm called (the Environment and health of the future) arranged by Läkartidningen and Medical doctors for the Environment (a special issue on the topic was published in Läkartidningen, with one article authored by Larsson and Christina Greco from the Swedish Veterinary Institute). Lina Gunnarsson gave the main talk during two consecutive days at the "Pharmaceutical days" for region Skåne, each attracting about 400 participants in March 2014. Larsson assisted the Swedish Department of Social Affairs and the Department of Environment the in a workshop in Brussels where a strategic agenda for the EU on pharmaceuticals in the environment was discussed. Here, we particularly raised the need to assess risks for antibiotic resistance associated with environmental emissions of antibiotics. Larsson also gave a presentation at the Nordicast meeting in Gothenburg, attracting clinical microbiologists from the Nordic countries. Larsson gave a presentation at the "National Life-science day" in Stockholm, attended by e.g entrepreneurs from various sectors. He also took part in the production of educational movies which now is part of a web-based environmental education course that all medical doctors in the Stockholm region have to pass. Larsson also talked about antibiotic resistance in the environment at a meeting at SwAM when a delegation from China visited Sweden. Finally, Larsson gave a presentation at "Kloka listan forum" in Stockholm.

Stockholm University

Project leader: Marlene Ågerstrand

Ågerstrand was invited to give presentations at: The Royal Society of Chemistry and Lancaster University workshop on Systematic review, UK; The US EPA IRIS-NRC Recommendations Workshop; The Swedish Agency for Marine and Water Managements seminar at the Water and Sanitation fair; The Swedish Chemicals Agency; The Norman network, France, and Almedalen, Sweden. Ågerstrand has also been updating the webpages www.mistraparma.se and www.scirap.org.

Project leader: Magnus Breitholtz

Since 2011, ITM has worked closely with the Sustainable Seas Initiative in Stockholm. The intention of this initiative is to bring together representatives from the world of research and business along with NGOs and the community at large, and thereby put further pressure on politicians. In 2014, ITM was responsible for a Sustainable Seas seminar, which was hosted by the Crown Princess and broadcasted on Swedish Television. Pharmaceuticals in

the environment was one of the main topics for the seminar. Pls. see more information above under Conferences and seminars/Almedalen.

Royal Institute of Technology (KTH)

The ongoing work with the mobile lab has raised an interest in media. An interview in Swedish Radio about treatment technologies and the status in the Baltic Sea was given i August. Interviews in newspaper articles were published in Ny Teknik and Uppsala Nya Tidning. On the web, Swedish Research Council published an interview in their Web magazine Curie.

In end of June / beginning of July two presentations on were given at Almedalen in Visby. Pls. see more information above under Conferences and seminars/Almedalen.

Treatment technologies and the project were presented for the staff at Käppala, Uppsala and Västerås WWTP. Visitors to Käppala WWTP were informed about the treatment project during the daily study tours.

Brunel University

Professor Sumpter had very regular contact, including meetings, with employees of a number of major pharmaceutical companies. He also advised an NGO, CHEMTrust, on its publication "Pharmaceuticals in the environment: a growing threat to our tap water and wildlife" (published December, 2014). He also contributed to a number of articles on the issue of pharmaceuticals in the environment that were published in the media. He contributed to a radio programme on this issue broadcast by the BBC. He attended meetings of the UK government and the European Union concerned with pharmaceuticals in the environment.

Staff

Karin Liljelund and Helene Hagerman (Trossa AB).

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