MistraPharma

Annual report 2013

Annual report for MistraPharma 2013

Again, it has been a year of high activity and important achievements!

At the policy level, a very important development was that the Governmental Bill, defining a platform for Swedish chemicals policy, includes the millstone target for increased environmental consideration in EU pharmaceutical legislation and internationally.With this decision, the Swedish Government has set a high level of ambition for Sweden in the work to develop improved EU – and international policies in this area. According to the Bill by 2020 decisions should have been made in the European Union or internationally which means that existing and possible new rules on medicinal products for human and veterinary use factor in environmental aspects to a greater extent. MistraPharma aims to be an active collaborator to support the Swedish Government in these efforts.

The research activities in the program are proceeding at "cruising speed", and a lot of important knowledge has been produced during the year. Below are some high-lights:

- Together with researchers at Karolinska Institutet a web-tool for reporting and evaluation of (eco)toxicity studies has been developed and made available free of charge at www.scirap.org. The aim of the criteria and the tool is to facilitate the use of non-standard data in environmental (and health) risk assessment. This tool is now used to develop risk assessment approaches based on non-standard data in collaboration with international experts.
- Through our collaboration with the Initiative Sustainable Seas, water samples were taken in the Baltic Sea by the ship Tre Kronor. All these samples have now been analyzed, i.e. in total 42 samples, taken from Halmstad to Haparanda. The results clearly show that pharmaceuticals

are present in detectable concentrations in the Baltic, both at coastal and open sea locations.

- Identification and evaluation pharmaceuticals of high concern have continued, and several studies are ongoing to increase our knowledge about bio-concentration processes. One interesting finding is that invertebrates bio-concentrate, and are affected by, anti-histamines and this has also implications for bio-magnification.
- The studies in frogs and fish have to a large extent been focused on the effects of progestins, which are synthetic versions of the endogenous hormone progesterone and common ingredients in contraceptive pills. Some very important findings have been made, showing that levonorgestrel and other progestins are potent disruptors of both female and male reproductive functions at environmentally relevant exposure concentrations.
- In the project addressing effects from mixtures of pharmaceuticals two in vivo experiments in fish have so far been done: one involved a binary mixture of the two active ingredients of the contraceptive 'Pill', and the other a binary mixture of a synthetic progestogen and a synthetic androgen. Preliminary data analyses indicate some extremely interesting conclusions. First that pharmaceuticals can interact, or not, differently, depending on the level of organisation of the effect. And second, that two pharmaceuticals can act completely independently at the molecular and biological level, even when present in a mixture. If we can substantiate this finding with other 'simple' mixtures of pharmaceuticals, a very important general principle will have been established.
- The pilot plant for waste water treatment has been constructed and is in full operation. The main focus is on ozone treatment and the use of activated carbon. Ozone can effectively remove pharmaceuticals, but at the same time it may produce transformation products that are equally or more toxic than the mother compounds. Currently, we are therefore studying ozone treatment in combination with activated carbon/sand filtration as final treatment steps. Wastewaters from the pilot plant (currently installed at Käppala), are therefore tested for toxicity using a combination of ecotoxicity tests and biochemical markers.
- Antibiotic-resistance has been studied in bacteria isolated from a treatment plant in India receiving process water from a large number of pharmaceutical industries. In these studies, all of the 93 investigated isolates were multi-resistant, and 86% were resistant to at least 20 out of 39 tested antibiotics. As much as 95% of the isolates carried integrons, a

genetic system allowing the bacteria collect arrays of resistance genes. As far as we know, this is the most extreme environment described, both with regards to multi-resistance as well as integrons.

- MistraPharma's work with networking and stakeholder communication has been highlighted in a new brochure: "MistraPharma – med fokus på kommunikation" (In Swedish). Mistra has used this as an inspiration for other Mistra programs.

I wish to thank all the people contributing to the MistraPharma program. Your dedication, generosity, collaborative attitudes forms the fundament of the program and is a key to its achievements! Furthermore, it should be noted that MistraPharma is not just a research program, it is a network of scientists and stakeholders and the success, and usefulness of our work build on the trust, respect and fruitful collaborations developed within this network. Therefore, my warmest thanks go also to our committed Program Board and Reference Group; Thank you!

Christina Rudén, programme director

Content

Programme structure 8

Financial report 10

Project reports

Umeå University XX

University of Gothenburg/
Chalmers University of Technology XX

Uppsala University XX

Stockholm University XX

Royal Institute of Technology (KTH) XX

Brunel University XX

Communication project XX

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
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 (130212, 130315, 130930, 131111).

Programme director

Christina Rudén

Commmunication manager

Karin Liljelund

Reference group

Alicja Andersson, Medical Products Agency
~~Camilla Berglund~~ 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
Kerstin Gustafsson, the Swedish Chemicals Agency
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~~ Sara Byfors, 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

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Financial Report

	Outcome 2013	Outcome 2012-2013
REVENUES		
Allocated funding from Mistra	15 188 105	26 671 349
Other revenues*	275 051	1 368 357
TOTAL REVENUES	**15 463 156**	**28 039 706**
COST		
Personnel costs	6 907 243	11 607 108
Travel expenses	242 619	421 142
Supplies	2 388 620	3 067 602
Depreciation	118 693	142 679
Other operating expenses	615 238	1 019 360
DIRECT COSTS	**10 272 413**	**16 257 891**
Overhead including premises costs	1 524 835	2 642 191
Costs including overhead	**11 797 248**	**18 900 082**
Purchased services	644 444	1 417 817
TOTAL COST	**12 441 692**	**20 317 899**
BALANCE	**7 526 728**	**12 159 912**
ALLOCATED FUNDS PHASE 2 2012-2015		**XX**
REMAINING FUNDS		**XX**

* 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 and Jerker Fick

Summary of completed research 2013

Evaluation of High Risk APIs

Identification and evaluation API's of high concern has continued during 2013. This work is still based on the fish plasma model, i.e. calculations based on the human therapeutic plasma concentration). First results from our extended bioconcentration study that was performed in 2009 at Gryab (Gothenburg) will be submitted shortly (Grabikova et al). This study focus on tissue distribution of antidepressants in muscle, brain, liver, and plasma taken simultaneously. One additional manuscripts focusing on the fish plasma model and the implications this model has on regulatory processes have been delayed and is planned to be submitted mid-2014. In addition to the controlled BCF-studies at waste water treatment plants, a 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 also planned to be submitted mid-2014. Two additional studies have been performed investigating bioconcentration in invertebrate aquatic species, one study, that was recently published, show that invertabrates bioconcentrate and are affected by anti-histamines (Jonsson et al 2014) and the other study show the importance of bioaccumulation (Brodin et al). As a measurement of persistency of API's, a study focusing on photo degradation experiments was done 2012 and the manuscript will be submitted shortly (Golokova et al). A novel approach to investigate persistency of API's was made possible by analyzing sea samples taken by the ship Tre Kronor during 2013. This study has already received a lot of attention and all samples have now been analyzed, including 13 samples taken by a PhD student at the Department of Chemistry in related research project (EcoChange). These 42 samples, taken from Halmstad to Haparanda, clearly show that API's are present in detectable concentrations in the Baltic, in both samples taken at coastal and open sea locations and a manuscript will be written during 2014.

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. This study has now been expanded and also include 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 waters and sediments at productions sites and reference sites in heavily polluted areas in Pakistan have been published (Khan et al 2013). An additional study with a similar set-up have been performed in River Stångån in Linköping have just been submitted (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

Results from the chemical oxidation experiments using ozone and hydrogen peroxide during MistraPharma Phase I (LU and DTU) have now been published (Antoniou et al 2013, Hey et al 2013). Two papers addressing pharmaceuticals in sludge treatments have also been published Davidson et al a and b 2013). One major topic during 2013 has been to support KTH to optimize and validate the ozone- and/or carbon-based tertiary treatment.

Analytical determinations

The development of an improved screening method for determination of +100 APIs has been concluded and a manuscript has been submitted (Lindberg et al). A sensitive and extended analytical method for progestines (LOQ at 0.01 ng/L) has been done and a manuscript will be written after a full validation has been made. The sample throughput, as support for the whole MistraPharma program, has been high and a total of about 1900 samples have been analyzed during 2013, excluding lab and field blanks, standards and QA / QC runs. These analyzes are distributed as follows; 693 analyzes for GU (flecainide, ketoprofen, NSAID and antibiotic studies), 540 analyzes for UmU (sewage water, photo-degradation and method development), 192 analyzes for KTH (wastewater treatment study) and 484 analyzes for UU (progestins).

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 2014

The studies of photo transformation/degradation will continue including search for transformation products, Jerker Fick will analyze samples from this study at the high resolution mass spectrometer at the lab of Dr Grabic in the Czech republic in April 2014. Environmental (matrix) factors of importance for photolysis will be investigated. Additional experiments regarding bioconcentration in aquatic species other than fish will be performed. 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 2014, 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 2013

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

Publications

Published manuscripts

Davidsson Å, Kjerstadius H, Haghighatafshar S, Fick J, Olsson M, Wachtmeister H, Eriksson E, la Cour Jansen J. 2014. Effect of anaerobic digestion at 35, 55 and 60°C on pharmaceuticals and organic contaminants Water Science and Technology In Press

Hey G, Vega SR, Fick J, Tysklind M, Ledin A, la Cour Jansen J, Andersen HR. 2014. Removal of pharmaceuticals in WWTP effluents by ozone and hydrogen peroxide. Water SA, 40, 165-174.

Jonsson M, Fick J, Klaminder J, Brodin T. 2014. Antihistamines and aquatic insects: bioaccumulation and impacts on behavior in damselfly larvae (Zygoptera). Science of the Total Environment, 472, 108-111.

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.

Antoniou MG, Hey G, Vega SR, Spiliotopoulou A, Fick J, Tysklind M, Ledin A, la Cour Jansen J, Andersen HR. 2013. Required ozone doses for removing pharmaceuticals from wastewater effluents. Science of the Total Environment, 456, 42-49.

Davidsson A, Eriksson E, Fick J. 2013. Ozonation and Thermal Pre-Treatment of Municipal Sewage Sludge-Implications for Toxicity and Methane Potential. Journal of Residuals Sciences & Technology 10(2), 85-91.

Khan GA, Berglund B, Khan KM, Lindgren PE, Fick J. 2013. Occurrence and abundance of antibiotics and resistance genes in rivers, canal and near drug formulation facilities − a study in Pakistan. PLoS ONE 8(6): e62712. doi:10.1371/journal.pone.0062712

Svensson J, Fick J, Brandt I, Brunström B. 2013. The progestin levonorgestrel is a potent androgen in the three-spined stickleback (Gasterosteus aculeatus). Environmental Science & Technology 47, 2043-2051.

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

Säfholm M., Jansson E., Fick J., Berg C. 2014. Antagonistic Mixture Effects of Levonorgestrel and Ethinylestradiol on Androgen Receptor mRNA expression during Sex Differentiation. *Submitted manuscript.*

Johnning A, Kristiansson E, Fick J, Weijdegård B, Larsson DGJ. 2014. High abundance of resistance mutations in gyrA and parC in bacterial communities sampled in both fluoroquinolone polluted and pristine environments. *Submitted manuscript.*

Lindberg RH, Östman M, Olofsson U, Grabic R, Fick J. 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. *Submitted manuscript.*

Säfholm M, Jansson E, Ribbenstedt A, Fick J, Berg C. 2014. Risks of hormonally active pharmaceuticals to amphibians: A growing concern regarding progestagens. *Submitted manuscript.*

Brodin T, Piovano S, Fick J, Klaminder J, Heynen M, Jonsson M. 2014. Ecosystem effects of pharmaceuticals in aquatic systems – impacts through behavioural modifications. *Submitted manuscript.*

Berglund B, Fick J, Lindgren PE. 2014. Detection and Quantification of Antibiotic Resistance Genes in Stångån River, Sweden. *Submitted manuscript.*

Golovko O, Fick J, Lindberg RH, Östman M, Grabic R. 2014. Photodegradation characteristics of pharmaceuticals in water under impact of UV and sunlight irradiation. *Manuscript in preparation.*

Publications associated projects

Östman M, Lindberg RH, Fick J. 2014. A Snapshot of the Illicit Drug Use in Sweden Acquired Through Sewage Water Analysis Science of the Total Environment, 472, 862-871.

Berglund B, Khan GA, Weisner SEB, Ehde PM, Fick J, Lindgren PE. 2014. Efficient removal of antibiotics in surface –flow constructed wetlands, with no observed impact on antibiotic resistance genes. Science of the Total Environment 476-477, 29-37.

Loos R, Carvalho R, Comero S, António DC, Locoro G, Tavazzi S, Paracchini B, Ghiani M, Lettieri T, Gawlik BM., Blaha L, Jarosova B, Voorspoels S, Haglund P, Fick J, Lindberg RH, Schwesig D. 2013. EU-wide monitoring survey on waste water treatment plant effluents. Water Research 47, 6475-6487.

Klaminder J, Fick J, Jonsson M, Sundelin A, Brodin T. 2013. Effects of a benzodiazepine (Oxazepam) on different life stages in Eurasian perch (Perca fluviatilis). *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.

Lindberg R, Sahlén K, Tysklind M. 2013. Occurance and distribution of synthetic organic substances in boreal coniferous forest soils fertilized with hygienized municipal sewage sludge. Antibiotics, 2, 352-366,.

Singer AC, Järhult J, Grabic R, Fedorova G, Khan GA, Fick J, Lindberg RH, Bowes MJ, Olsen B, Söderström H. 2013. Compliance to Oseltamivir among two populations in Oxfordshire, United Kingdom affected by Influenza A(H1N1)pdm09, November 2009 − a wastewater epidemiology study. PLoS ONE 8(4): e60221. doi:10.1371/journal.pone. 0060221.

Fedorova G. Randak T, Lindberg RH, Grabic R. 2013. Comparison of the quantitative performance of a Q-Exactive high-resolution mass spectrometer with that of a triple quadrupole tandem mass spectrometer for the analysis of illicit drugs in wastewater. Rapid Communications in Mass Spectrometry. 27(15): 1751-1762.

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.

Daneshvar A, Prévost M, Fick J, Kronberg L, Weyhenmeyer GA. 2013 Natural waters remove pharmaceuticals faster than nutrients. *Submitted manuscript.*

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.*

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.

Conference contributions

Gunnarsson L, Fick J, Gräns A, Axelsson M, Larsson JDG. How does sewage effluent exposure affect the pharmacokinetics of non-steroidal anti-inflammatory drugs (NSAIDs) in fish? 23:th SETAC Europe Meeting, Glasgow, UK 12-16 May, 2013.

Gunnarsson L, Fick J, Axelsson M, Hofgaard Brattström L, Gräns A, Larsson JDG. 2013. How does sewage-effluent exposure affect the pharmacokinetics of non-steroid anti-inflammatory drugs in fish? 34:th Annual SETAC Meeting, Nashville, USA 17-21 November, 2013.

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 2013

Between January 2013 and now, the Gothenburg team published 7 original research articles on pharmaceuticals and/or antibiotic resistance in the environment, 7 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 and ongoing studies.

In 2013 we published two studies on antibiotic-resistant bacteria in bacteria isolated from a treatment plant in India receiving process water from a large number of pharmaceutical industries (Marathe et al, 2013; Johnning et al 2013). In the Marathe study, we showed that all of the 93 investigated isolates were multi-resistant, and 86% were resistant to at least 20 out of 39 tested antibiotics. As much as 95% carried integrons, a genetic system allowing the bacteria collect arrays of resistance genes. As far as we know, this is the most extreme environment described, both with regards to multiresistance as well as integrons. The result shows that environments with very high levels of antibiotics, such as industrial treatment plants, provide exceptional opportunities for the development of multi-resistant bacteria, and that integrons provide an important underlying mechanism. In the Johnning study, we sequenced the entire genome of an almost completely multiresistant bacterium isolated from the same plant. By comparing genotype and phenotype, we could attribute most of the acquired resistances to specific mobile genes and/or to specific mutations in target genes for the antibiotics. By doing this we could show how bacteria can evolve to adapt to such extreme selections pressures.

Larsson has co-authored four reviews on the environmental dimension of antibiotic resistance in 2013 (Pruden et al, 2013; Gaze et al, 2013; Ashbolt et al, 2013; Finley et al, 2013), all published in journals with relatively high impact factors (6.8-9.4). The Ashbolt paper deals specifically with how we

could go about to perform a "human health risk assessment" (HHRA) for environmental development and transfer of antibiotic resistance, pointing out critical points and knowledge needed. The Pruden paper is about management options for reducing the release of antibiotics and antibiotic resistance genes to the environment, covering different emissions routes such as municipal and industrial wastewater, animal farming etc. Larsson had a leading role (shared first author and corresponding author) in this paper, which has already received much attention, and a Chinese translation was recently published to reach a wider, global audience. These four reviews represent an example were academia have actively worked together with both industry and regulators to reach common grounds on various aspects needed for both risk assessment and management.

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. DNA sequencing and the first preliminary analyses of resistance genes was completed in 2013. The development of a manually curated, in-house antibiotic resistance gene database by the Kristiansson group was a prerequisite for this achievement. The preliminary analyses reveal a wide range of antibiotic resistance genes, but the final statistical analyses are ongoing. It is however clear already that, overall, there are great similarities between the three plants in how the resistance gene abundances change between different steps of the treatment processes. In a parallel project funded by FORMAS, we developed a database for metal and biocide resistance genes (Pal et al, 2014), which will allow us to explore the sequence data generated in an even more comprehensive way.

Plans for 2014

We will finalize the paper on antibiotic resistance genes in Swedish sewage treatment plants, and also take samples to study how resistance gene frequencies change with additional treatment (ozone, activated carbon). We are working on finalizing several papers on fish exposed to different pharmaceuticals, including NSAIDs and corticosteroids. We have completed the analyses of how the production site of the API for products on the Swedish market compare with the price for the final products, placing the results in light of environmental performance and corruption data from the production countries. Writing up the study still remains. We will also perform an experiment where we compare different methods to establish a selective concentration for an antibiotic, funded by the strategic reserve.

Staff

In the laboratory of Joakim Larsson, Carolin Rutgersson successfully defended her PhD during 2013 and is currently working as a research within the project. PhD student Johan Bengtsson successfully defended his halftime control during 2013 and his dissertation is planned for 2015. PhD student Anna Johnning will defend her thesis in April 2014 and continue with a postdoc at Chalmers. Researchers Dr Carl-Fredrik Flach postdoctors Dr Lina Gunnarsson and Dr Sara Lundström work in the project part-time. Dr Carl-Johan Svensson and Kim Andreas Aebelö, both amanuenses from the medical doctors programme, technician Birgitta Weidegård and Master student Malin Thoudal also work part-time within MistraPharma. Joakim Larsson was appointed Professor in Environmental Pharmacology during 2013. In September 2013, the entire Larsson laboratory moved from the Institute of Neuroscience and Physiology to the Institute of Biomedicine, department of Infectious diseases, both within the Sahlgrenska Academy at the University of Gothenburg. At the Chalmers node, docent Erik Kristiansson and PhD student Fredrik Boulund both work part-time within MistraPharma. Fredrik defended his licentiate degree during 2013.

Publications

Published manuscripts

Shanmugam G, Sampath S, Selvaraj KK, Larsson DGJ, Ramaswamy BR. 2014. Non-steroidal anti inflammatory drugs in Indian rivers. Environmental Science and Pollution Research 21:921-931. doi:10.1007/s11356-013-1957-6

Flach CF, Boulund F, Kristiansson E, Larsson DGJ. 2013. Functional verification of computationally predicted qnr genes. Annals of Clinical Microbiology and Antimicrobials. 12:34 doi:10.1186/1476-0711-12-34.

Johnning A, Moore ERB, Svensson-Stadler L, Shouche YS, Larsson DGJ, Kristiansson E. 2013. The acquired genetic mechanisms of a multi-resistant bacterium isolated from a treatment plant receiving wastewater from antibiotic production. Appl. Environ. Microbiol., 79(23):7256. doi:10.1128/AEM.02141-13.

Marathe NP, Regina VR, Walujkar SA, Charan SS, Moore ERB, Charan SS, Moore ERB, Larsson DGJ, Shouche YS. 2013. A Treatment Plant Receiving Waste Water from Multiple Bulk Drug Manufacturers Is a Reservoir for Highly Multi-Drug Resistant Integron-Bearing Bacteria. PLoS ONE 8(10): e77310. doi:10.1371/journal.pone.0077310

Kristiansson E, Österlund T, Gunnarsson G, Arne G, Larsson DGJ, Nerman O. 2013. A novel method for cross-species gene expression analysis. BMC Bioinformatics. 14:70. doi: 10.1186/1471-2105-14-70.

Rutgersson C, Gunnarsson L, Kristiansson E, Larsson DGJ. 2013. Oral exposure to industrial effluent with exceptionally high levels of drugs does not indicate acute toxic effects in rats. Environmental Toxicology and Chemistry. 32:577–584. doi: 10.1002/etc.2105.

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Boulund F, Johnning A, Pereira M, Larsson DGJ, Kristiansson, E. A method for identification of quinolone antibiotic resistance (qnr) genes in fragmented nucleotide sequence data, BMC Genomics, 13:694, 2012.

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

Larsson DGJ. Pollution from drug manufacturing: review and perspectives. Invited review, *submitted to Philosophical Transactions of the Royal Society B.*

Flach, C-F., Johnning, A., Nilsson, I., Kristiansson, E., Smalla, K. & Larsson, D. G. J. Isolation of novel broad host fluoroquinolone resistance plasmids from an antibiotic polluted lake. *Manuscript.*

Johnning, A., Kristiansson, E., Fick, J., Weijdegård, B., & Larsson, D. G. J. High frequencies of resistance mutations in gyrA and parC in bacterial communities sampled in both quinolone polluted and pristine environments. *Manuscript.*

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 and Greco C. 2014. Great drugs in the wrong place: risks for environmental effects and resistance promotion. Läkartidningen. Accepted for publication.

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Reports

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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

Publications associated projects

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Selvaraj KK, Shanmugam G, Sampath S, Larsson DGJ, Ramaswamy BR. (2013) GC-MS determination of bisphenol A and alkylphenol ethoxylates in the river water from India and their ecotoxicological risk assessment. Ecotoxicology and Environmental Safety. doi:10.1016/j.ecoenv.2013.09.006.

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

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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

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 Odonotology-programme, Nursing programmes and more. Teaching has extended to nurses and medical practitioners at the Nordic School for Public Health (NHV).

Uppsala University

Project leader: Ingvar Brandt

Summary of completed research 2013

The effect studies in frogs and fish have to a large extent been focussed on progestins, which are synthetic versions of the endogenous hormone progesterone and common ingredients in contraceptive pills. Some very important findings have been made, showing that levonorgestrel and other progestins are potent disruptors of both female and male reproductive functions at environmentally relevant exposure concentrations. On February 14, Moa Säfholm defended her PhD thesis entitled "Developmental and reproductive Toxicity of Progestagens in the Xenopus (Silurana) tropicalis Test System". On February 7, Johan Svensson defended his licenthiate thesis entitled "Androgenic Effects of the Progestin Levonorgestrel in Three-spined Stickleback (Gasterosteus aculeatus)".

The amphibian facility has been upgraded and a new re-circulation aquarium system installed. A new life-cycle study to examine flutamide has been initiated, exposure during the tadpole stage completed and samples collected at the juvenile stage. A manuscript on a study of combined exposures to a progestin and estrogen has been submitted. A complementary study on reproductive effects of norethindrone and progesterone in adult female amphibians has been carried out, and a paper submitted. An in vitro method to study effects of APIs on egg maturation in Xenopus tropicalis is currently being set up.

The common three-spined stickleback (Gasterosteus aculeatus) has recently been introduced in the project as a model for endocrine disruption in fish. Male stickleback kidneys produce spiggin, a glue-like protein used in nest building. Spiggin production is governed by androgens and spiggin production in females serves as the best known biomarker for androgen exposure in fish. In this project females were exposed to levonorgestrel (LNG) via the water for three weeks after which effects on spiggin biomarkers and egg yolk protein production were evaluated. Male sticklebacks that were in the final stage of a breeding period were exposed to various concentrations of LNG for six weeks under winter conditions, after which reproductive status was evaluated. In female sticklebacks, LNG induced spiggin production in the kidneys and suppressed the production of egg yolk proteins in the liver.

In males, LNG inhibited the post-breeding regression of breeding colours and spiggin production, as well as the resumption of sperm production; thus LNG functionally inhibits the natural transition from breeding into non-breeding condition. Suppression of egg yolk protein production in females and disruption of the male reproductive cycle as shown in our two papers could severely affect natural stickleback populations. Some of the present effects occurred well within the range of environmental LNG levels, and may therefore occur in progestin-contaminated waters. In conclusion, our results establish LNG as a highly potent androgenic pollutant of environmental concern, and support the contention that reproductive impairments in fish caused by progestins could to a significant degree be mediated by their androgenic properties.

On May 28, Kai Gao defended his PhD thesis entitled " Basal and Pollutant-induced Expression of CYP1A, 1B and 1C isoforms in Fish: Implications for Biomonitoring". In this work several new pollutant-responsive genes were evaluated with regard to their usefulness as biomarkers for exposure to different classes of pollutants in fish, particularly the CYP1A, 1B and 1C genes. One of the purposes of this work was to design suitable biomarkers to be used in the testing of improved sewage treatment technologies developed by Berndt Björlenius, KTH. Using these and other molecular and enzymatic biomarkers, a fist study in Käppala has been completed. The results show that effluent water from the Käppala STP was further purified both by addition of a carbon filter step and an ozonation step. The biological effects measured were more pronounced in gill than in liver tissue, indicating that bioactive pollutants remaining in the effluent were degraded by first pass metabolism in the fish body. As a supplement to these studies, the CYP-inhibitory activity of azole-related fungicidal drugs has been examined, using a new in vitro test system based on dissected gill filaments from rainbow trout. These results show that mixtures of azoles interact by additivity in fish gills.

Plans for 2014

A complementary study on endpoints for progestagenic activity in amphibian tadpoles will be carried out. Negative data on male reproductive toxicity of LNG together with assessment of distribution and target organs binding for LNG (autoradiographic study) in frogs will be published. A full life cycle study on developmental reproductive toxicity of flutamide and linuron will be finalized, including complete fertility tests, dissections, and sampling of adult frogs. A manuscript on effects of flutamide on gonadal differentiation and mRNA expression during sex differentiation is being planned. Installment of new frog tank systems will be finalized.

Comparative studies and mixture studies of various progestins plus the endogenous hormone progesteron (also an environmental pollutant) will be carried out. Zebrafish embryos will be exposed to various concentrations of LNG from fertilization to 80 day post fertilization. Sex ratios will be determined histologically, and transcript levels of key genes involved in sex differentiation measured. Exposure will start in March and finish in June. A pilot study was finished in December 2013, showing that a high concentration of LNG generated 100% males, compared to 40% males in the control. Using the female stickleback model of our first paper, progestins of differing androgenicity will be compared with regards to spiggin induction and vitellogenin suppression. Additive effects will also be measured. The study will be conducted in the fall of 2014.

As a continuation of the Käppala investigation a follow-up study will be carried out at the Uppsala STP, using the mobile STP unit developed by KTH.

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
Kai Gao, PhD student, fish project
Erika Jansson, PhD student, frog project
Maria Jönsson, Assistant professor, STP-fish project
Ylva Carlsson, Anton Ribbenstedt, Andreas Carlsson,
MSc students, frog project
Viktor Tsiamis, MSc student, STP project
Moa Säfholm, PhD student, frog project
Johan Svensson, PhD student, fish project

Publications

Published manuscripts

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

Säfholm, M., Jansson, E., Fick, J., Berg, C. Antagonistic Mixture Effects of Levonorgestrel and Ethinylestradiol on Androgen Receptor mRNA Expression during Sex Differentiation. *In preparation*.

Säfholm M, Jansson E, Ribbenstedt A, Fick J, Berg C. Risks of hormonally active pharmaceuticals to amphibians: A growing concern regarding progestagens. *Submitted*.

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.

Doctoral Thesis

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

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

Licentiate Thesis

Johan Svensson; Androgenic Effects of the Progestin Levonorgestrel in Three-spined Stickleback (Gasterosteus aculeatus). Completed in December 2013; defended 7 February 2014.

Master Thesis

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

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.

Stockholm University

Project leader: Marlene Ågerstrand

Summary of completed research 2013

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

Together with researchers and risk assessors at the Swiss Centre for Applied Ecotoxicology and the Dutch RIVM we have been conducting a ring test using our reliability and relevance evaluation criteria (the CRED-method). The ring test was divided into two parts; first the risk assessors were asked to evaluate ecotoxicity studies using the currently recommended method by Klimisch et al. (1997), and then by using the newly developed CRED-method. 75 risk assessors from Europe, Asia and North America participated in the study. The result from comparison is in favour of the CRED-method, both when it comes to the risk assessors' perception of the two methods as well as the actual evaluation results. Currently, four manuscripts are prepared.

We have also organized two workshops called "Closing the Gap Between Academic Research and Regulatory Risk Assessment of Chemicals", one at the SETAC conference in Glasgow and one at the EUROTOX conference in Interlaken. The workshops were well attended with between 130 and 170 participants. The workshops also opened up for future collaborations with editors of scientific journals regarding reporting recommendations for (eco) toxicity studies.

Together with researchers at IMM, Karolinska Institutet and ITM, Stockholm University we have developed a web-tool for reporting and evaluating of (eco)toxicity studies. The project is called Science in Risk Assessment and Policy (SciRAP), and the web-address is www.scirap.org. On the webpage our criteria for reporting and evaluating (eco)toxicity studies are available free of charge, together with a colour coding tool that provides the risk assessors with an overview of the reliability evaluation results. A manuscript presenting the tool is submitted for publication.

Weight of evidence

We have started a new project concerning weight of evidence in risk assessment. This is done in collaboration with researchers at the Institute of Environmental Medicine at Karolinska Institutet and the Department of Materials and Environmental Chemistry at Stockholm University. Currently we are doing a literature review with focus on guidance documents for risk assessment. The next step is to organize two workshops with leading international experts within this area. The workshops will be executed with additional funds from Mistra.

Suggestion for improvement of the EMA-legislation

Together with researchers within MistraPharma we are writing a paper concerning improvements of the current legislation for environmental risk assessment of pharmaceutical substances. A first draft of the manuscript has been prepared and is currently under discussion and revision.

Plans for 2014

In 2014 we will continue working with evaluation of data for use in risk assessment, focusing on a weight of evidence approach. We will continue the collaborations we have and developing new ones with scientific journals and regulators. We will organize international and national activities such as work-shops and other meetings. The manuscripts currently in preparation will be finalized and submitted for publication.

Staff

In 2013, the following personnel have been directly involved in the project:
- Christina Rudén
- Marlene Ågerstrand
- Magnus Breitholtz

Publications

Published manuscripts

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

Molander L, Ågerstrand M, Beronius A, Hanberg A, Rudén C. Science in Risk Assessment and Policy (SciRAP) – an online resource for evaluating and reporting (eco)toxicity studies. Submitted for publication.

Ågerstrand M, Kase R, Korkaric M, Moermond C. Towards more consistency and transparency in risk assessment. Four manuscripts in preparation.

Ågerstrand M, Breitholtz M, Berg C, Brandt I, Brunström B, Fick J, Gunnarsson L, Kristiansson E, Larsson J, Tysklind M, Rudén C. How to improve the European Medicines Agency's guideline on the environmental risk assessment of medicinal products for human use – Making use of available research data. Manuscript in preparation.

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).

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.

Conference presentations

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

Teaching - undergraduates and practioners

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".

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.

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Stockholm University

Project leader: Magnus Breitholtz

Summary of completed research 2013

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. Currently, we are therefore studying ozone treatment in combination with activated carbon/sand filtration as final treatment steps. In the end of 2013, we have received different wastewaters from the mobile MistraPharma pilot plant (currently installed at Käppala), which we are testing using a combination of ecotoxicity test and biochemical markers.

To be able to screen a large number of wastewater streams in parallel, it is both from a logistical and financial point of view impossible to use long-term tests focused on adverse endpoints. Still, APIs are designed to be biologically active in humans and it is known that some of the human drug targets have been evolutionary conserved, and hence are present also in lower aquatic organisms, such as crustaceans and fish. It is therefore important to try to find and adopt rapid yet sensitive biomarker techniques that are indicative of adverse effects in these groups of species. To complement our ecotoxicity tests focussing on more adverse endpoints, we have therefore invested most of the time and efforts in 2013 to develop and adopt a battery of sensitive 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. She defended her licentiate thesis in 2013 and recently also submitted two papers on this topic (see publication list). 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.

As a spin-off project to our MistraPharma project, we have submitted several applications the last year in order to attract funding for developing a methodology for sensitive effect screening of chemical mixtures in surface water and sediment. We believe that this is a research area of great potential and with high relevance for mixture toxicity research, regulatory risk assessment and environmental monitoring of APIs and other organic contaminants. Briefly, the basic idea is to use silicone-based equilibrium passive samplers to sample organic chemicals present in the aquatic environment. The passive samplers will thereafter be extracted and the extract loaded to silicone cast in micro-wells for re-creation of the environmental chemical mixture in bioassays. Passive dosing systems may also facilitate experimental testing of poorly soluble substances in water-based bioassays. For instance, in a Master student project we recently showed that in a 6-week chronic endpoint bioassay, a stable water concentration of a hydrophobic test chemical was maintained throughout the experiment when using the silicone passive dosing system. Currently, we have a new Master student who is using the passive dosing approach to test synthetic mixtures of APIs, which we hope to submit as a scientific paper later this year.

Another spin-off project is focusing on bacteria-host interactions as a consequence of antibiotic exposure in crustaceans. 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 con-

comitant 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. In 2012, we reported dramatic effects of antibiotics (ciprofloxacine, trimethoprime and sulfomethoxazole) on microbiome community in the harpacticoid copepod Nitocra spinipes (Edlund et al., 2012). In a follow-up study (Gorokhova et al, submitted manuscript) we recently exposed D. magna to antibiotics with the objectives 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. 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. Briefly, 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. 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 2014

The plan for 2014 is to continue our evaluations of different wastewater treatment technologies. We will test a second round of wastewaters from Käppala and also wastewaters from two additional sewage treatment plants. As part of the Käppala work, we have recently hired a Master student from Germany who in more detail will study transformation products from APIs in the pilot plant. Three relevant APIs will be selected and spiked to both tap water and wastewater. Growth and reproduction effects in algae and D. magna will be supplemented by analytical determination of transformation products, both before and after ozonation.

We will also continue to develop and apply biochemical tools to unravel mechanistic effects of APIs in D. magna and to develop and validate population-level mathematical models for improved environmental risk assessment. As part of the latter task, Elin Lundström will finalize the two manuscripts that are in preparation and defend her doctoral thesis in May this year. This work started already in phase 1 but has been delayed since Elin Lundström, the PhD student working on this topic, has been on parental leave twice over the years the program has been ongoing.

Staff

In 2013, the following personnel have been involved in the project:
- Magnus Breitholtz
- Elena Gorokhova
- Elin Lundström
- Sara Furuhagen
- Birgitta Liewenborg
- Karin Ek

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 Belleza, E., Breitholtz, M., Gorokhova E. Are pharmaceuticals with evolutionary conserved molecular drug targets more potent to cause effects in non-target organisms? Submitted to PLOS ONE.

Furuhagen, S., Liewenborg, B., Breitholtz, M., Gorokhova, E. Feeding activity and xenobiotics modulate oxidative status in Daphnia magna: implications for ecotoxicological testing. Submitted to Environmental Science and Technology.

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

Lundström Belleza, E., Brinkmann, M., Preuss, T.G., Breitholtz, M. Less becomes more- population modeling from standard ecotoxicity data. Manuscript in preparation.

Lundström Belleza, E., Breitholtz, M. Free sex and population growth in Nitocra spinipes. Manuscript in preparation.

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.

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

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

Master Thesis

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

Conference contributions

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.

Royal Institute of Technology (KTH)

Project leaders: Gen Larsson
Assistant project leader: Berndt Björlenius

Summary of completed research 2013

KTH/Industrial biotechnology .....

Plans for 2014

XXX.....

Staff

XXXX.....

Publications

Brunel University

Project leaders: John Sumpter

Summary of completed research 2013

XXXX....

Plans for 2014

XXXX....

Staff

XXX...

Publications

XXX....

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 16
The meeting was held at Käppala sewage treatment plant. Berndt Björlenius gave the reference group a guided tour which included the mobile pilot plant of MistraPharma. Bengt Mattson, LIF/Pfizer, presented and discussed tasks 7.2 (environmental assessment model of pharmaceutical products) and the proposals from Sofia Wallström in the environmental report from the Pharmaceutical and Pharmacy Inquiry. Magnus Breitholtz, Stockholm University, presented a first proposal for an improved risk assessment model for pharmaceuticals that MistraPharma is developing. 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. The meeting was very constructive and fruitful.

October 21
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 communication project presented the work within the working group for a continuation of MistraPharma after 2015. The meeting ended with a joint dinner with the researchers and the program's board of directors.

Information

Brochure

Mistra wanted to highlight the communication work within MistraPharma as a good example and a broschure was produced, "MistraPharma - med fokus på kommunikation". Mistra have used this as an inspiration for other Mistra programs.

Article

MistraPharma has written an article in the information journal of the Medical Products Agency (Tidningen Blå) which is distributed to all physicians, medical students, nurses and midwives with the right to prescribe, veterinarians and others. It is published six times per year and the edition of the printed version of the journal is 64 000 copies.

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 and seminars

MistraPharma helped EUFEPS (European Federation for Pharmaceutical Sciences) arrange the conference "Human Pharmaceuticals in the Environment – Challenges in research and the need for societal action" which was held in Uppsala 27th of June.

MistraPharma helped the Initiative Sustainable Seas (Hållbara hav) to arranged a seminar to throw light into the challenges that threaten the welfare and wellbeing of the Baltic Sea. The seminar, which was held on the 5th of September, attended Crown Princess Victoria as well as representa-

tives from academia, industry, government, NGOs and the media. Jerker Fick, Marlene Ågerstrand, Christina Rudén,Magnus Breitholtz and Berndt Björlenius participated and presented their research.

During the second half of 2013 MistraPharma started planning for multiple seminars at Almedalen week 2014, 30th of June to 2nd of July, together with the Initiative Sustainable Seas (Hållbara hav), ITM and the Baltic Sea Center at Stockholm University, The Swedish Water & Wastewater Association and the Medical Products Agency.

MistraPharma after 2015

MistraPharma board has decided to appoint a working group to work on the issue of how MistraPharma could continue after 2015. This committee has been led by the communication project and three meetings have been held during 2013; 17th of May, 9th of September and 15th of October.

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

Fick J. Invited speaker at a press conference at the AAAS meeting in Boston, USA 2013 .

Tysklind M. Invited speaker. ”Miljögifter i slam och utgående vatten”. Seminarium: Slam och kretslopp – slam som produktionsresurs i svenskt jord- och skogsbruk? Kungl. Skogs- och Lantbruksakademien. Stockholm, 27 Februari, 2013

Fick J. Invited speaker at the JRC, Ispra site, Institute for Environment and Sustainability, seminar on pharmaceuticals in the environment, Italy, 11 April, 2013 .

Fick J. Invited speaker at "Inititivet Hållbara hav" meeting in Stockholm, Sweden, 5 September, 2013.

Tysklind M. "Invited speker. Miljögifter i Östersjön – igår, idag och i framtiden". Havsmiljökonferens Örnsköldsvik, 23 September, 2013.

Fick J. Invited speaker at "NO-biennalen" meeting in Umeå, Sweden, 25 September, 2013.

Fick J. Invited speaker at "Bok- och biblioteksmässan" meeting in Gothenburg, Sweden, 28 September, 2013.

Tysklind M. Fick J. "Forskning om gamla och nya organiska miljögifter". Seminarium - Umeå Marina Forskningscentrum (UMF), Umeå universitet, Norrbyn, 30 September, 2013.

University of Gothenburg

The Gothenburg team has engaged extensively in communication activities with stakeholders and the public during 2013. 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). LIF has provided the Ministry for Health and Social Affairs with a report, and a proposal from the ministry has been open for comments during 2013 (SOU 2013:23 – Ersättning vid läkemedelsskador och miljöhänsyn i läkemedelsförmånerna, S 2013/3153/FS). Dr Lina Gunnarsson and Larsson have authored a formal remittance. Larsson has together with Charlotte Unger taken part in the development of environmental procurement criteria for the United Nations (UN), in particular the World Health Organization (WHO) and the United Nations Environmental Development Programme (UNEP). Larsson is a member of the Swedish expert panel for the Joint Programming Initiative on Antimicrobial Resistance (JPI-AMR). In this role, he authored the Swedish remittance for the part relating to the antibiotics and antibiotic-resistance external environment, which led to important changes in the European strategic research agenda which was recently published (http://www.jpiamr.eu/). Together with Jerker Fick, Larsson contributed to a TV documentary by Folke Rydén on pharmaceuticals in the Baltic Sea broadcasted in Vetenskapens Värld. Larsson and Carl-Fredrik Flach also participated in a longer documentary film on pharmaceuticals in our water by Peter Podjavorsek, broadcasted in Swedish SVT2, in the Swedish Kunskapskanalen as well as in German and French TV during 2013.

Stockholm University

Project leader: Marlene Ågerstrand

During 2013 Ågerstrand and Rudén participated in Sustainable Seas seminar "Chemicals and the marine environment" (http://www.hallbarahav.nu/seminarierna), the FILM&SCIENCE festival at Nobel museum in Stockholm, and the FEMREP conference in Uppsala.

Rudén has had several meetings with the Swedish Chemicals Agency and the Swedish EPA concerning different aspects of chemicals risk management. Rudén participated in Folke Rydén's documentary "The Toxic Compromise". Rudén has made presentations of MistraPharma to ITM's Board, and to the Ministry of Health and Social Affairs. Rudén has also continued her work as a member of the ECHA's Management Board and the Swedish Chemicals Agency's Supervisory Council ("Insynsråd"), provided support to the Swedish MPA, and followed up the result of the "Miljömålsberedningen". Rudén has also participated in interviews in several Swedish newspapers and other media.

Ågerstrand participated at Stockholm County Councils workshop for sustainable use of pharmaceuticals with the talk: "To identify risks. Experiences from MistraPharma". She also gave a presentation at Gävleborgs County Councils environmental day. Ågerstrand has also given seminars at the Swedish Chemicals Agency and at ITM, Stockholm University. Ågerstrand has, together with Helene Hagerman, developed and updated MistraPharma's new webpage (www.mistrapharma.se). Currently there are in average 230 unique visitors per month. Ågerstrand has also participated in interviews in Chemical Watch.

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 2013, ITM and other universities involved in MistraPharma were responsible for a Sustainable Seas seminar, which was hosted by the Crown Princess and broadcasted on Swedish Television. Pharmaceuticals in the environment was the main topic for the seminar.

Breitholtz M (2013) Östersjöns status? Invited speaker at network meeting in the in the presence of the Swedish Crown Princess, politicians, representatives from the commercial and the industrial life as well as independent

non-governmental organizations. 28th of August 2013 at the City Hall of Stockholm.

Breitholtz M, Rudén C (2013) Kemikalier i havsmiljön - Tillsammans hittar vi lösningar! Invited speaker at Sustainable seas (Hållbara Hav) seminar in the presence of the Swedish Crown Princess. Broadcasted on Swedish Television. 5th of September 2013 at Kastellholmen, Stockholm, Sweden.

Uppsala University

Berg C. Pharmaceuticals in the environment – a threat to reproduction in amphibians? The Environmental Chemistry Day, the Swedish Chemical Society, 2013. Invited speaker.

Berg C. Workshop on endocrine disrupting chemicals, Swedish chemicals Agency, 2013. Invited speaker.

Berg C. Intervjued for the article Hormonfria skydd in Fältbiologen, No 2, 2013. Fältbiologerna/Nature and Youth Sweden.

Berg C. Meeting with Alicja Andersson and Kia Salin at the Medical Products Agency, regarding environmental risks of hormones used in contraceptives, August 2013.

Brandt I. Workshop on endocrine disrupting chemicals, Swedish chemicals Agency, 2013. Invited speaker.

Bergman Å, Andersson AM, Becher G, van den Berg M, Blumberg B, Bjerregaard P, Bornehag CG, Bornman R, Brandt I, Brian JV, Casey SC, Fowler PA, Frouin H, Giudice LC, Iguchi T, Hass U, Jobling S, Juul A, Kidd KA, Kortenkamp A, Lind M, Martin OV, Muir D, Ochieng R, Olea N, Norrgren L, Ropstad E, Ross PS, Rudén C, Scheringer M, Skakkebaek NE, Söder O, Sonnenschein C, Soto A, Swan S, Toppari J, Tyler CR, Vandenberg LN, Vinggaard AM, Wiberg K, Zoeller RT. Science and policy on endocrine disrupters must not be mixed: a reply to a "common sense"intervention by toxicology journal editors. Environ Health. 2013 Aug 27;12:69.

Staff

Karin Liljelund and Helene Hagerman (Trossa AB)