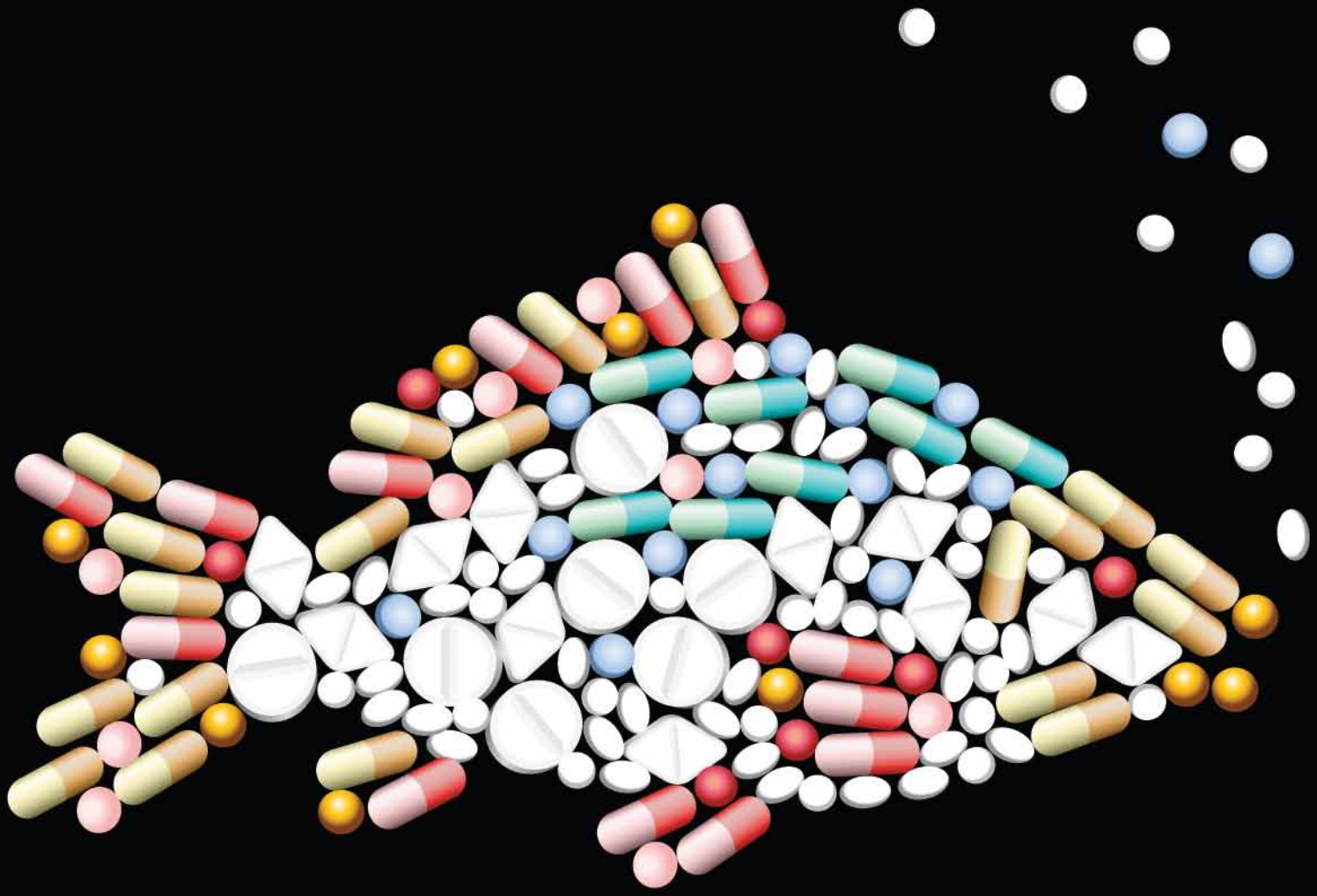


Identification and Reduction of Environmental Risks Caused by Human Pharmaceuticals

MistraPharma Research 2008–2015

Final Report

It has been a genuine pleasure and joy to serve as the Programme Director of MistraPharma, and all the people that contributed to its success will always have a special place in my heart!

Preface

This report summarizes the main findings and achievements made within the MistraPharma research program (www.mistrapharma.se). The program started in January 2008 and ended in December 2015 and the research was generously financed by the Swedish Foundation for Strategic Environmental Research – Mistra (www.mistra.org).

The program included the following partners: The University of Gothenburg, Stockholm university, Umeå university, Uppsala university, the Royal Institute of Technology – KTH (second phase), Brunel University (second phase) and the Technical University in Lund – LTH (first phase). Trossa AB was responsible for the program's stakeholder communication.

The Program Board and the Reference Group included representatives from all the major stakeholders in Sweden. The Board and the Reference Group were actively involved in supporting and developing the program during all eight years. Their important contributions are gratefully acknowledged.

One of the special features of MistraPharma was its emphasis on stakeholder communication. The communication activities were coordinated by Karin Liljelund at Trossa AB. She furthermore had a significant role in the overall running of the program. Karin's expertise and professionalism were certainly a key to the program's successful and fruitful interactions with Swedish stakeholders.

Finally, my warmest thanks to all the students, researchers and technicians that have contributed to the program during the years, in particular the PIs. Together I think we succeeded in creating a research environment that combined scientific excellence with a true interdisciplinary approach and integration of the different sub-projects. And at the same time we had a really nice time together!

It has been a genuine pleasure and joy to serve as the Programme Director of MistraPharma, and all the people that contributed to its success will always have a special place in my heart!

Christina Rudén
Programme Director

Content

Identify high risk Active Pharmaceutical Ingredients

Professor Ingvar Brandt
Uppsala University

Associate professor Jerker Fick
Umeå University

Introduction

When the MistraPharma program was in the planning stage, evidence had accumulated that certain human pharmaceuticals (Active Pharmaceutical Ingredients; APIs) may pass sewage treatment plants (STPs) and be disseminated in recipient waters. The presence of a pharmaceutical in the environment does, however, not per se imply that it that it may give rise to pharmacological or adverse responses in aquatic wildlife at environmentally relevant concentrations. Except for the contraceptive drug ethinylestradiol (EE2), which had been extensively studied at the time, little information on the health risks posed by pharmaceuticals on aquatic wildlife was available. Today, some 300 APIs are known to enter the aquatic environment (Hughes et al., 2013; Lindberg et al., 2014; Loos et al., 2009; Petrie et al., 2015), and the list is growing. There is consequently a major need to monitor the release of human APIs in the aquatic environment, and to determine if and how they can impact aquatic organisms at a range of concentrations in the water. Adverse effects on reproduction, development and growth are particularly important endpoints in an ecological context. Pharmaceuticals are of vital importance for human health, it is not an option to refrain from using them. In rare cases a drug may be substituted for an environmentally more friendly alternative, e.g. in case of certain non-steroidal anti-inflammatory drugs such as diclofenac. In most cases this is not possible. It is therefore highly important to identify high risk APIs and develop practical measures and management strategies to reduce the risks posed by exposure to these APIs, both with regard to human health (exposure via drinking water) and the environment. This challenge is being increasingly recognized by pharmaceutical industry, regulatory authorities, the health care system, and the scientific community. Assessing hazards and risks posed by pharmaceuticals in the environment is different from assessing chemicals in general. Unlike most chemicals, APIs (and pesticides) are designed to interact specifically with a variety of molecular targets (receptors, enzymes, ion channels, etc.) to evoke a desired biological response in the body. Likewise, interactions with unwanted targets need to be as low as possible to avoid or reduce side-effects and toxicity. Both preclinical development and safety assessment of a new pharmaceutical include studies on experimental animals such as rodents, rabbits and dogs. The value of these test systems is based on the experience that a pharmacological action and many unwanted adverse effect in humans do frequently occur also in these species (although major species differences may become evident). Recent research shows that many molecular targets are evolutionary old and conserved among species (Brown et al., 2014; Gunnarsson et al., 2008). Numerous human pharmaceuticals are therefore active also in lower vertebrates such as birds, amphibians and fish, and occasionally also in invertebrates. Biological effects of APIs are consequently expected to occur also in aquatic wildlife, provided that

exposure is sufficiently high. It should further be kept in mind that most APIs are designed to be rapidly eliminated and/or excreted from the body (half-lives from hours to days), in order to avoid overdosing and enable to reduce side effects and toxicity by reducing the dose. Compared to "conventional" contaminants such as PCBs and PBDEs, the half-life of pharmaceuticals in the body is therefore very short, and it can be a challenge to convince stakeholders that human pharmaceuticals released in the environment may reach concentrations high enough to pose a threat to sensitive aquatic species. It is therefore important to note that the half-life of a pharmaceutical in water and sediment may be very long compared to that in in humans and wildlife, thus sustaining a continuous exposure of aquatic wildlife to APIs that are readily eliminated from the human and fish body.

Prioritization for monitoring and effect characterization

With more than 6000 pharmaceuticals on the global market, it is necessary define procedures for selecting those compounds which should be included in environmental monitoring schemes. Likewise, it is necessary to define procedures for selecting APIs to be examined for biological activity in vivo at concentrations close to those found in the environment. Several ranking schemes, based on different types of data, often combined, with expert judgement and/or case-by-case assessment, have been proposed (Berninger and Brooks, 2010; Besse and Garric, 2008; Jerker Fick et al., 2010b; Huggett et al., 2003; Roos et al., 2012). For highly potent hormonal APIs, decisions for testing in resource-heavy test systems need a high degree of expert judgement. One reason for that is that the sensitivity of the analytical techniques available is not high enough to analyse the APIs at biologically active concentrations. In the MistraPharma program we have used both of analytical information (when available) and expert judgement to prioritize APIs for reproductive and developmental toxicity studies in amphibians and fish.

A useful approach for priority setting ("the Fish Plasma Model") has been suggested by Huggett et al. 2003. The plasma model is based on the assumption that two species sharing the same drug target are expected to develop pharmacological effects at more or less the same plasma concentrations. The fish plasma model generates a concentration ratio (CR) between the human therapeutic plasma concentrations (H_TPC) and a measured, or a theoretically derived, fish steady state plasma concentration ($F_{ss}PC$). When the concentration ratio is < 1, the plasma concentration in an exposed fish is equal to, or higher, than the plasma concentration that gives rise to a pharmacological response of an API in humans. In this situation the fish should be expected to show a pharmacological response. A lower ratio reflects a higher risk. MistraPharma has applied the fish plasma model to predict the risks to fish that human pharmaceuticals may pose. The plasma concentration approach is particularly useful since it allows a theoretical risk to be calculated for a great majority of pharmaceuticals. The human therapeutic plasma concentration is well defined for most APIs and is available in the literature. Based on such calculations it is possible to predict if an exposed fish in a specific region would be at risk to be affected. In one study, surface water concentrations required to theoretically produce a pharmacological response were calculated for 500 pharmaceuticals (Fick et al., 2010b). These surface water concentrations, defined as "critical environmental concentrations, CECs", were derived from theoretically predicted FSSPCs and published human therapeutic plasma concentrations. By combining predicted or measured environmental concentrations with CEC values for these pharmaceuticals, it would be possible to predict or calculate CRs in a specific geographical region.

MistraPharma has evaluated and compared the fish plasma model with other prioritization schemes (Roos et al., 2012). Nine schemes, both risk and hazard-based, were evaluated and used to rank 582 APIs. The similarities and differences in overall ranking results and input data were compared and the ranking of seven well-studied APIs was evaluated. The study showed that the hazard-based methods were more successful in correctly ranking the well-studied APIs, but the fish plasma model also showed a high success rate.
Analyses showed that the availability of input data varies significantly; data such as logP are available for most API while information on environmental concentrations and bioconcentrations are still scarce. One MistraPharma study investigated bioconcentration in fish exposed to sewage effluent and compared measured levels in fish plasma with predicted levels based on lipophilicity (Fick et al., 2010a). Concentrations of 25 pharmaceuticals in plasma of rainbow trout exposed to effluents for 14 days were investigated in three sites in Sweden. The anticontraceptive progestagen levonorgestrel was measured in fish blood plasma at concentrations exceeding the human therapeutic plasma concentration. Totally 16 pharmaceuticals were found at concentrations higher than 1/1000 of the human therapeutic plasma concentration. This investigation also showed that the majority of the pharmaceuticals gave a reasonably good agreement between theoretically calculated and experimentally determined plasma concentrations. Other research groups have evaluated the read-across hypothesis with similar results (Rand-Weaver et al., 2013). It is obvious that the fish plasma model is not suitable to assess effects of APIs directed towards microorganisms and parasites, particularly because the sensitivity of these organisms towards APIs may show great variation. In MistraPharma phase 2, a separate work package was included to increase understanding of antibiotic resistance promotion in the environment (see chapter 2).

Furthermore, most prioritization schemes do not account for extreme pollution loads that may occur at pharmaceutical production and formulation facilities. Pollution from API production units have previously not been considered as significant sources of release of APIs in the environment. Several recent studies show that many production sites globally are contributing to environmental pollution levels far above those previously reported (Fick et al. 2010; Larsson, 2014; Larsson et al., 2007; Li et al., 2009; Phillips et al., 2010).

Key findings in amphibians and fish

Despite that the dissemination of pharmaceuticals in the environment has been a cause of concern for more than two decades, little information has been available to confirm that APIs may pose a threat to aquatic wildlife in highly exposed areas. Major efforts have been devoted to studying the oral contraceptive ethinylestradiol (EE2) while most other APIs have been given less attention. When starting the MistraPharma program, we set the goal to identify at least one pharmaceutical with a potency and efficacy similar to that of EE2, i.e. high enough to pose a threat to aquatic wildlife reproduction and development. During the course of these studies, we and others observed that the oral anticontraceptive levonorgestrel (LNG) may pose an ecotoxicological threat to lower aquatic vertebrates, of a magnitude similar to that of EE2. Moreover, as opposed to EE2, there are several progestagens with a similar effect profile as LNG in amphibians and fish. Given that these APIs act on the same hormone receptors, we provide evidence to suggest that they may act in concert. We consequently propose that progestagens may give rise to additive, so called cocktail (or mixture) effects in the environment. Papers demonstrating additivity of progestagens are in progress.

Our work has provided considerable new information also about other types of APIs (se publication list). Developmental effects of EE2 was initially examined in amphibians. Previous results in different amphibian species had shown that larval exposure to high EE2 concentrations (ug/l concentration range) can induce female-biased sex ratios male-to-female sex reversal, indicating male-to-female sex reversal (Kloas et al. 1999; Mackenzie et al. 2003). A life-cycle study conducted at much lower EE2 concentrations (ng/l range) showed not only induce permanently skewed sex ratios towards female frogs, but also impaired spermatogenesis and reduced fertility in the males as they reached sexual maturity (Gyllenhammar et al. 2009). A significant proportion of the adult EE2-exposed phenotypic females lacked oviducts, making them sterile. It was concluded that testicular development and differentiation of the Mullerian ducts (progenitors of the oviducts in other vertebrates except fish) are sensitive targets for developmental estrogen exposure in frogs.

Another important part of the project has been to develop molecular biomarkers in fish. These biomarkers do not measure adverse effects in the fish but rather initiating molecular events that might result in downstream overt toxicity. The main purpose of the biomarker studies was to evaluate to which extent fish are exposed to different classes of pollutants when they are caged in recipient waters or allowed to swim in water sampled from the environment. In a first study, the biomarkers were used to characterize the "biologic" activity of highly diluted water sampled at the Patanchero water treatment plant in India, containing very high concentrations of human APIs (Beijer et al. 2013). We measured mRNA transcript expression of CYP genes and a number of other biomarker genes. The conclusion of the results was that an altered function of the CYP system caused by highly diluted water may affect various physiological functions including the regulation of endogenous hormone levels in fish. The panel of biomarkers employed has also been used to evaluate new STP purification procedures developed by the KTH group.

Effects of progestins in amphibians

To expand from the EE2 studies in frog, we undertook to examine whether the progestagen LNG can interfere with reproduction and development in amphibians. Progestagenic effects were examined both after exposure of adult frogs, and of tadpoles (Figure 1, experimental protocol). In a series of studies following exposure of adult frogs to LNG, norethindron (NET) and progesterone, the ovaries were evaluated histologically to quantify eggs in different stages of maturation. mRNA levels for vitellogenin, and estrogen/androgenreceptors were also recorded. The results revealed that all compounds inhibited egg maturation, particularly the transition from the previtellogenic stage to the stage when vitellogenin is incorporated in the eggs. The formation of vitellogenic oocytes was inhibited at environmentally relevant concentrations, i.e. 1-10 ng/L (Säfholm et al., 2012; 2014). These findings are in agreement with an earlier study showing that 1 ng/l of LNG inhibited egg production in fish (Zellinger et al., 1999). The observation that LNG and NET indeed inhibited egg formation at concentrations as low as 1 ng/l is remarkable, particularly because the therapeutic plasma concentration of LNG in a woman is more than thousand times higher. The results strongly suggest that progestagens may pose a risk for impaired fertility in wild amphibians living in progestin-contaminated water bodies. Adult frogs exposed to LNG during the larval stage also displayed reduced oocyte maturation in the ovaries (Figure 1, experimental protocol). Another notable finding in these frogs was that they completely lacked or had underdeveloped oviducts. These results suggest that larval exposure to LNG targeted both Mullerian duct differentiation and oogenesis, resulting in permanently impaired fertility. Mating experiments with adult males and females from this experiment indeed confirmed that the females were sterile (Kvarnryd et al. 2011). A follow up-study revealed that larval LNG exposure caused an increased expression of the membrane bound progesterone receptor which persisted long after exposure was discontinued, suggesting that this receptor may be involved in the mechanism for developmental reproductive toxicity in amphibians (Säfholm et al 2015). In conclusion, progestagens such as LNG and NET reduced oocyte maturation in adult females and gave rise to Mullerian duct dysgenesis and reduced egg maturation in adults exposed only during the tadpole stage. These findings imply that a full life-cycle study is required to disclose the severe consequences of developmental exposure to this type of APIs (Säfholm et al., 2014). Our results showing that the progestagens, LNG, NET and the natural hormone progesterone (which is also present in STP effluents) inhibited egg development by targeting formation of vitellogenic eggs suggest that these compounds are likely to give additive effects in aquatic wildlife (Säfholm et al 2014).

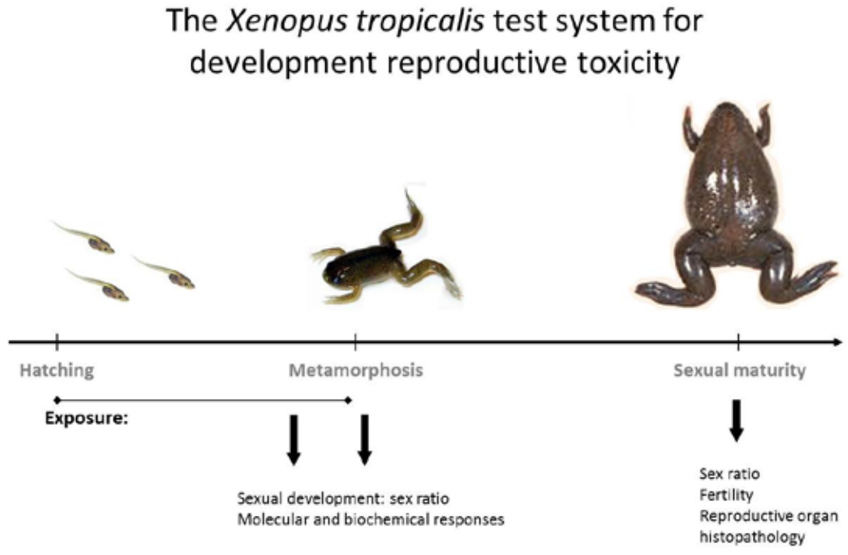


Figure 1. Experimental protocol for developmental toxicity studies in frogs. Larvae are exposed to an API from shortly after hatching until meamorphosis. A variety of endpoints are examined at metaborphosis and in the sexually mature adults.

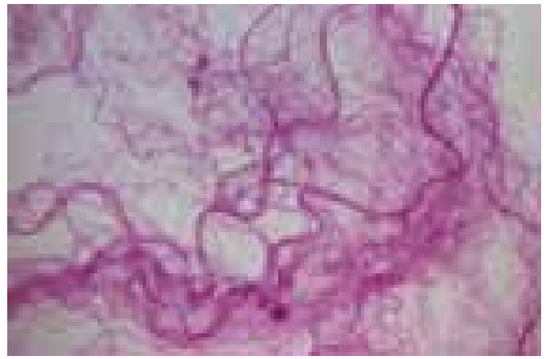


Figure 2. Coils of spiggin aggregates collected from water were LNG-exposed female sticklebacks were swimming. Spiggin is normally secreted by the kidneys of reproducing males and subseguently used as a glue for nest building. PAS staining.

Effect of progestins in fish

Most progestagens are not pure agonists on the progesterone receptor but may interact with other steroid hormone receptors as well. Their ability to stimulate the androgen receptor is of particular interest. To explore the androgenic potency of progestagens in fish, we took advantage of the common three-spined stickleback as a test system for androgenicity. During the reproductive season male sticklebacks produce a protein called spiggin in their kidneys. Spiggin is a glue-like protein that the males use for nest building following secretion via the urine (Figure 2). Spiggin production is regulated by androgens and in females it serves as the best known biomarker for androgen exposure in fish. When female sticklebacks were exposed to levonorgestrel (LNG) via the water for three weeks, an up to more than 100 000-fold induction of spiggin mRNA production was observed in their kidneys. This finding shows that LNG is

a highly efficient androgen in fish (Svensson et al. 2013), presumably the most efficient and potent environmental androgen demonstrated to date. In another experiment, male sticklebacks that were in the final stage of their breeding season were exposed to various concentrations of LNG for six weeks. The reproductive status was then evaluated. In these males, both the post-breeding regression of breeding colours and the spiggin production were resumed. Even more striking was the finding that LNG also blocked the seasonal inhibition of sperm production in these fish (Svensson et al., 2014). These findings show that LNG inhibits the seasonal transition from a breeding into a non-breeding physiology in male stickleback. Some of the effects occurred within the range of environmental LNG concentrations, and may therefore occur in progestin-contaminated waters.

A follow-up study in zebrafish showed 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 in the water. The concomitant observation that the natural hormone progesterone did not affect the sex ratio supports the conclusion that the androgenic activity of progestins could be particularly important in fish. Interestingly, however, both LNG and progesterone were found to induce precocious puberty along with altered expressions of gonadotrophic hormone mRNA transcripts in the pituitary of zebrafish (Svensson et al., 2015).

In conclusion, our results have established LNG and other progestagens as a highly potent androgenic pollutants of environmental concern. The results support the contention that reproductive impairments in fish caused by LNG and other progestins could to a significant degree be mediated by their androgenic properties.

Other deliverables

The subproject has so far resulted in five doctoral theses and one licentiate thesis that have been defended at Uppsala University. A final doctoral thesis will be defended in early June 2016. In addition, 14 master theses have been completed and defended.

Key publications

Fick, J., Lindberg, R.H., Parkkonen, J., Arvidsson, B., Tysklind, M., Larsson, D.G.J., 2010a. Therapeutic Levels of Levonorgestrel Detected in Blood Plasma of Fish: Results from Screening Rainbow Trout Exposed to Treated Sewage Effluents. Environ. Sci. Technol. 44, 2661–2666.

Fick, J., Lindberg, R.H., Tysklind, M., Larsson, D.G.J., 2010b. Predicted critical environmental concentrations for 500 pharmaceuticals. Regul. Toxicol. Pharmacol. 58, 516–523.

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

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

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

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 161: 146–153.

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.

Säfholm M, Ribbenstedt A, Fick J, Berg C. 2014. Risks of hormonally active pharmaceuticals to amphibians: A growing concern regarding progestagens. Phil. Trans. R. Soc. B. 369:20130577.

Roos, V., Gunnarsson, L., Fick, J., Larsson, D.G.J., Ruden, C., 2012. Prioritising pharmaceuticals for environmental risk assessment: Towards adequate and feasible first-tier selection. Sci. Total Environ. 421, 102–110.

References

Berninger, J.P., Brooks, B.W., 2010. Leveraging mammalian pharmaceutical toxicology and pharmacology data to predict chronic fish responses to pharmaceuticals. Toxicology Letters 193, 69–78.

Besse, J.-P., Garric, J., 2008. Human pharmaceuticals in surface waters 176, 104–123.

Boxall, A.B.A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., Ankley, G.T., Beazley, K.F., Belanger, S.E., Berninger, J.P., Carriquiriborde, P., Coors, A., DeLeo, P.C., Dyer, S.D., Ericson, J.F., Gagne, F., Giesy, J.P., Gouin, T., Hallstrom, L., Karlsson, M.V., Larsson, D.G.J., Lazorchak, J.M., Mastrocco, F., McLaughlin, A., McMaster, M.E., Meyerhoff, R.D., Moore, R., Parrott, J.L., Snape, J.R., Murray-Smith, R., Servos, M.R., Sibley, P.K., Straub, J.O., Szabo, N.D., Topp, E., Tetreault, G.R., Trudeau, V.L., Van Der Kraak, G., 2012. Pharmaceuticals and Personal Care Products in the Environment: What Are the Big Questions? Environmental Health Perspectives 120, 1221–1229.

Brown, A.R., Gunnarsson, L., Kristiansson, E., Tyler, C.R., 2014. Assessing variation in the potential susceptibility of fish to pharmaceuticals, considering evolutionary differences in their physiology and ecology. Philosophical Transactions of the Royal Society of London B: Biological Sciences 369, 20130576.

Fick, J., Lindberg, R.H., Parkkonen, J., Arvidsson, B., Tysklind, M., Larsson, D.G.J., 2010a. Therapeutic Levels of Levonorgestrel Detected in Blood Plasma of Fish: Results from Screening Rainbow Trout Exposed to Treated Sewage Effluents. Environ. Sci. Technol. 44, 2661–2666.

Fick, J., Lindberg, R.H., Tysklind, M., Larsson, D.G.J., 2010b. Predicted critical environmental concentrations for 500 pharmaceuticals. Regul. Toxicol. Pharmacol. 58, 516–523.

Fick, J., Söderström, H., Lindberg, R.H., Phan, C., Tysklind, M., Larsson, D.G., 2010. Contamination of surface, ground, and drinking water from pharmaceutical production. Environ. Toxicol. Chem. 28, 2522–2527.

Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., Larsson, D.G.J., 2008. Evolutionary Conservation of Human Drug Targets in Organisms used for Environmental Risk Assessments 42, 5807–5813.

Huggett, D.B., Cook, J.C., Ericson, J.F., Williams, R.T., 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish 9, 1789–1799.

Hughes, S.R., Kay, P., Brown, L.E., 2013. Global Synthesis and Critical Evaluation of Pharmaceutical Data Sets Collected from River Systems. Environ. Sci. Technol. 47, 661–677.

Klaminder, J., Jonsson, M., Fick, J., Sundelin, A., Brodin, T., 2014. The conceptual imperfection of aquatic risk assessment tests: highlighting the need for tests designed to detect therapeutic effects of pharmaceutical contaminants. Environmental Research Letters 9, 084003.

Larsson, D.G.J., 2014. Pollution from drug manufacturing: review and perspectives. Philosophical Transactions of the Royal Society B-Biological Sciences 369, 20130571.
Larsson, D.G.J., de Pedro, C., Paxeus, N., 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. J. Hazard. Mater. 148, 751–755.

Li, D., Yang, M., Hu, J., Ren, L., Zhang, Y., Li, K., 2009. Determination and fate of oxytetracycline and related compounds in oxytetracycline production wastewater and the receiving river 27, 80–86.

Lindberg, R.H., 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. Water Research 58, 221–229.
Loos, R., Gawlik, B.M., Locoro, G., Rimaviciute, E., Contini, S., Bidoglio, G., 2009. EU-wide survey of polar organic persistent pollutants in

Understanding promotion of antibiotic resistance in the environment

Professor D G Joakim Larsson
University of Gothenburg

Associate Professor Erik Kristiansson
Chalmers University of Technology

The global societal challenge of antibiotic resistance, and the role of the environment

More than 8 billion doses of antibiotics are used every year, a treatment that often makes the difference between life and death. Meanwhile, infections with multi-resistant bacteria increase rapidly worldwide, now claiming an estimated 700,000 lives per year. This loss of antibiotic efficacy severely limits our ability to cure infections and to provide medical treatment in cases where risks of infections are high, including surgery, neonatal care and immunosuppressive therapy. Infections with resistant bacteria thereby complicate treatment, lead to substantial increases in health care and societal costs and, ultimately, increased morbidity and mortality. The emergence of multi-resistant pathogens has become a global challenge with bacteria rapidly crossing national borders. The way forward is not obvious; there are both scientific and economic hurdles for developing novel antibiotics, while, at the same time, improper use of existing antibiotics rapidly drives resistance progression. In fact, each dose of an antibiotic may select for resistant bacteria, resulting in reduced future value of the drug for everyone. Hence, limiting resistance represents a social dilemma wherein private interests are at odds with collective interests, leading to suboptimal outcomes from a societal point of view. For these reasons, the World Health Organization (WHO) identifies antibiotic resistance as a global challenge so serious that it threatens the fundamental achievement of modern medicine.

The WHO as well as many national bodies, strongly advocate a "one-health-approach" to tackle antibiotic resistance, i.e. an approach that addresses both humans, animals and the external environment. The reason for this is the interconnectedness of these compartments, and that bacteria evolve and spread with limited respect for such perceived borders. The external environment plays two critical roles in antibiotic resistance. One role is in the transmission of (resistant) pathogens, often via fecal contamination of water. In many low income countries, sewage infrastructure is limited or even absent. There, reducing environmental transmission is a highly critical control point. The other role is in the emergence of resistance in pathogens, where harmless environmental bacteria can serve as a source for novel resistance genes or resistance elements that can be transferred horizontally to pathogens, which has happened many times in the past. Such transfer events are boosted by a selection pressure from antibiotics.

A major overarching knowledge gap has been, and still is, to what extent environmental pollution with antibiotics contribute to resistance transmission and to the emergence of resistance in pathogens. What are the concentrations that select for resistance? Are antibiotic concentrations found inside or downstream from sewage treatment plants a problem or should we perhaps focus on other sources? With regard to manufacturing discharges – how widespread is the problem? Do such discharges promote resistance? Can we find evidence that resistance, developed in antibiotic-polluted environments, have the ability to spread to human pathogens? What are the gaps in regulation and management and how could these gaps be filled? These were some of the questions we asked ourselves when we started to work with antibiotic resistance in the MistraPharma programme.

What did we show?

Starting off within an idea support programme from Mistra to Joakim Larsson, and extending into the larger MistraPharma programme, we have shown in a series of papers that the largest emissions of pharmaceuticals on earth do not arise from usage, but from manufacturing discharges (Larsson et al. 2007, Larsson 2008, Fick et al. 2009, Kristiansson et al. 2011, Janzon et al. 2012, Larsson 2014). Concentrations found in treated effluent or downstream from an industrial treatment plant in India reached up to mg/L levels, with the broad-spectrum antibiotic ciprofloxacin at the top with 31 mg/L (Larsson et al. 2007). The drugs have also contaminated river sediment (Kristiansson et al. 2011), ground and well water in surrounding villages (Fick et al. 2009), and irrigated farmland (Rutgersson et al. 2014). The concentrations found in contaminated effluent were considerably higher than the levels found in the blood of patients taking the medicine. Accordingly, we showed in a series of papers that the effluent was highly toxic to a range of organisms (but not acutely toxic to rats if ingested in small amounts) (Larsson et al. 2007, Carlsson et al. 2009, Gunnarsson et al. 2009, Rutgersson et al. 2013). Although the toxicity of the effluent represents a serious finding, the most worrying results were the effects on antibiotic resistance in exposed microbial communities. Inside the treatment plant, all bacteria we have isolated are multi-resistant, with a typical bacterium resistant to 30 out of 39 tested antibiotics (Marathe et al. 2013) and some are resistant to almost every tested antibiotic (Johnning et al. 2013). To the best of our knowledge, this is the most extreme environment in terms of multi-resistance ever reported. The levels of resistance genes in the receiving river were exceptionally high, also 17 km downstream from the plant (Kristiansson et al. 2011). And in a nearby lake, where industrial effluents are dumped, we found high numbers of both resistant bacteria (Flach et al. 2015) and diverse antibiotic resistance genes (Bengtsson-Palme et al. 2014). Additionally, the resistance genes were accompanied by a similarly high diversity of genetic elements that can favour mobility of resistance (Bengtsson-Palme et al. 2014). Accordingly, when we cultured antibiotic-susceptible E.coli bacteria (a model for an important human pathogen) together with environmental bacteria from the contaminated lake, various resistance plasmids were transferred to the E.coli, making them instantly resistant to a range of antibiotics (Flach et al. 2015). In a couple of other studies, we have shown that Swedish visi-

> *In a lake where industrial effluents are dumped, we found high numbers of both resistant bacteria and diverse antibiotic resistance genes*
>
> Joakim Larsson

tors to India (and Africa) bring home resistance genes, resistance mutations and resistant bacteria in their gut flora, making the resistance situation in India our problem as well (Bengtsson-Palme et al. 2015, Johnning et al. 2015). Another reason to be concerned about the pollution situation in India is that they produce drugs for the Swedish market, thus we save money on the environmental shortcuts taken by the producers, a quite questionable situation from a moral standpoint (Larsson and Fick 2009).

We have also investigated how the abundances and types of resistance genes change through the treatment process of regular sewage treatment works in Sweden (to be communicated). To evaluate the use of advanced treatment options, we have commenced to study the potential benefits with full-scale ozonation concerning antibiotic resistance within the Knivsta project (to be communicated). Selective concentrations of 111 antibiotics have been theoretically estimated from available data on concentrations know to inhibit growth of pathogens (Bengtsson-Palme and Larsson 2016). This has been empirically verified for tetracycline, showing that a very low concentration (1 microgram/L, found in regular sewage treatment plants) can select for resistance. We also showed that a somewhat higher concentration (10 micrograms/L) which is still 150 times the concentration that completely inhibits growth, can induce the actual transfer process of resistance between bacteria (Jutkina et al. 2016).

Finally we have developed methodology to identify and quantify hundreds of different resistance genes in parallel using next generation DNA sequencing (Kristiansson et al. 2011, Bengtsson-Palme et al. 2014, Bengtsson-Palme et al. 2015, Boulund et al. 2015, Jonsson et al. 2016, Lundström et al. 2016). Another software was developed that allow parallel assessment of the taxonomic composition of bacteria in such data sets (Bengtsson-Palme et al. 2015). We have also used our developed skills in working with large-scale DNA sequencing data to identify novel resistance genes (Boulund et al. 2012, Flach et al. 2013).

Where did it take us?

Our research in India changed the previously completely dominating view that pharmaceuticals in the environment arise from the use and excretion of drugs, described in hundreds of earlier scientific publications. Drugs, including antibiotics, apparently can have effect throughout their lifecycle, i.e. before, during and after usage! The levels we found also fundamentally changed the general perception of the risks involved, and expanded the risks to a much larger set of pharmaceuticals (Larsson 2014). Finally, it put focus on a different set of management needs that extend well beyond any national or even continental borders, and made pharmaceuticals in the environment a truly global challenge (Larsson 2010, Boxall et al. 2012, Larsson 2014, Ågerstrand et al. 2015). Given the exceptionally high concentration of antibiotics and the clear effects on resistance, it also turned some the focus in the field from strictly environmental effects (such as feminization of fish, population collapses of birds) to also include a clear human health risk.

Management needs and the overarching challenges with antibiotics in the environment have been addressed in a number of publications by us (Larsson et al. 2007, Larsson and Fick 2009, Larsson 2010, Ashbolt et al. 2013, Finley et al. 2013, Gaze et al. 2013, Pruden et al. 2013, Graham et al. 2014, Larsson and Lööf 2014, Sandegren et al. 2015, Bengtsson-Palme and Larsson 2016). Since the start of MistraPharma, we have participated in about 100 different seminars, meetings and other events involving politicians, stakeholders from different sectors etc. We have also contributed to a series of radio programmes, documentary films for cinemas, TV and the Internet with broad national and international coverage (http://biomedicine.gu.se/ominst/avd/infektion/forskare/joakim_larsson/prizes_lectures_and_media). A very

large number of news articles have been written about our research, many with direct support from us. Altogether, this has increased awareness and contributed to a number of societal management initiatives. Some of these are listed below:

- Initiatives from the Swedish county councils to include emission control during manufacturing in the procurement process of pharmaceuticals. This is a world-leading initiative.
- Similar initiatives are currently in consideration by the United Nations Development Programme (UNDP).
- A proposal (SOU 2013:23) to the Swedish parliament to include pollution control (and not only price) in the assignment of the "product-of-the-month" within the generic substitution system. This would create economic incentives to invest in greener production.
- A commission by the British Government, addressing the global challenges with antibiotic resistance, has recently proposed direct regulation of emissions of antibiotics from drug manufacturing world-wide (AMR Review 2015), using the discharge limits proposed by Bengtsson-Palme and Larsson (2016).
- An intensified work by several pharmaceutical industries to work with improvements of environmental standards in their production chains.
- Inclusion of control of manufacturing emissions in other countries within Sweden's National Pharmaceutical Strategy (NLS 2016).
- A stronger focus on environmental aspects within the Swedish Authorities' Strategy to combat antibiotic resistance (PHA 2015).
- A proposal from Sweden to EU to amend the Framework for Good Manufacturing Practice with environmental criteria.

What is ahead?

Many of the initiatives above need to be continuously promoted to create a real effect, and fortunately the challenge and responsibility has in most cases been taken on by other actors in society. As researchers, we still think we can contribute with scientific input to regulations, procurement criteria etc, and to educate.

There are of course also still unresolved research needs. We are currently leading other research programmes dealing with the environmental dimensions of antibiotic resistance (http://biomedicine.gu.se/ominst/avd/infektion/forskare/joakim_larsson). These include FORMAS and VR projects aiming to better understand if there is indeed resistance selection occurring at the relatively low concentrations found in regular sewage treatment plants or surface waters. Another large project, Interact (www.interact.gu.se), addresses the co-selective potential of metals and biocides that can act in concert with antibiotics. As the most worrying resistance threats are those genes that we still do not know are resistance genes (Bengtsson-Palme and Larsson 2015), we have a project exploring environmental reservoirs for resistance genes called "NoCure". The resistance challenge is, however, much wider than the environmental aspects, and even within the environmental dimensions, we need expertise from various disciplines to efficiently address the problems. We are therefore exceptionally grateful that the University of Gothenburg has allocated about 50 million SEK 2016-2022 to an interdisciplinary Centre for Antibiotic Resistance Research at University of Gothenburg (CARe). The centre will be led by Larsson and at the start it involves more than 80 researchers and PhD students from six faculties. Our vision is to limit mortality, morbidity and socioeconomic costs related to antibiotic resistance on a global scale. To stand a better chance to contribute to these visions, we would very much like to continue the valuable interactions with the network of stakeholders generated during the MistraPharma programme.

Acknowledgement

We have both had the privilege to do research together with a large number of talented and dedicated co-workers. Without them, there would not have been much research at all done. The collaborations within the MistraPharma network, including both researchers and members of the reference group, have also been crucial. In addition to Mistra, who has funded the research, we have had joint funding from several other sources, out of which FORMAS, VR, SIDA, University of Gothenburg, Västra Götalandsregionen, Chalmers University of Technology, The Wallenberg foundation and Adlerbertska forskningsstiftelsen have made the greatest contributions.

Key publications

Bengtsson-Palme J, Larsson DGJ. (2016) Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International. 86:140-149. doi:10.1016/j.envint.2015.10.015

Bengtsson-Palme J, Larsson DGJ. (2015) Antibiotic resistance genes in the environment: prioritizing risks. Nature Reviews Microbiology. 13, 396. doi:10.1038/nrmicro3399-c-1

Flach CF, Johnning A, Nilsson I, Smalla K, Kristiansson E, Larsson DGJ. (2015) Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. Journal of Antimicrobial Chemotherapy. 70(10):2709-2717. doi: 10.1093/jac/dkv167

Larsson DGJ. (2014) Pollution from drug manufacturing: review and perspectives. Philosophical Transactions of the Royal Society B, 369: 20130571. http://dx.doi.org/10.1098/rstb.2013.0571

Lundström SV, Östman M, Bengtsson-Palme J, Rutgersson C, Thoudal M, Sircar T, Blanck H, Eriksson KM, Tysklind M, Flach CF, Larsson DGJ. (2016) Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. Science of the Total Environment. . 553: 587-595.

References

AMR Review. Antimicrobials in agriculture and the environment: reducing unnecessary use and waste. http://amr-review.org/Publications

Ashbolt NJ, Amezquita A, Backhaus T, Borriello P, Brandt KK, Collignon P, Coors A, Finley R, Gaze WH, Heberer T, Lawrence JR, Larsson DGJ, McEwen SA, Ryan JJ, Schonfeld J, Silley P, Snape JR, Van den Eede C, Topp E. Human Health Risk Assessment (HHRA) for environmental development and transfer of antibiotic resistance. 2013. Environ. Health Perspect. 121(9): 993-1001.

Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, Larsson DGJ, Johansson A. The human gut microbiome as a transporter of antibiotic resistance genes between continents. 2015. Antimicrob. Agents Chemother. doi:10.1128/AAC.00933-15.

Bengtsson-Palme J, Boulund F, Fick J, Kristiansson E, Larsson DGJ. Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. 2014. Front. Microbiol. 5. doi:10.3389/fmicb.2014.00648.

Bengtsson-Palme J, Hartmann M, Eriksson KM, Pal C, Thorell K, Larsson DGJ, Nilsson RH. METAXA2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. 2015. Mol. Ecol. Resour. 15(6): 1403-1414.

Bengtsson-Palme J, Larsson DGJ. Antibiotic resistance genes in the environment: prioritizing risks. 2015. 13. doi:10.1038/nrmicro3399-c1.

Bengtsson-Palme J, Larsson DGJ. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. 2016. Environ. Int. 86: 140-149.

Boulund F, Johnning A, Pereira MB, Larsson DGJ, Kristiansson E. A novel method to discover fluoroquinolone antibiotic resistance (qnr) genes in fragmented nucleotide sequences. 2012. BMC Genomics 13. doi:10.1186/1471-2164-13-695.

Boulund F, Sjögren A, Kristiansson E. Tentacle: distributed quantification of genes in metagenomes. 2015. Gigascience 4. doi:10.1186/s13742-015-0078-1.

Boxall AB, Rudd MA, Brooks BW, Caldwell DJ, Choi K, Hickmann S, Innes E, Ostapyk K, Staveley JP, Verslycke T, Ankley GT, Beazley KF, Belanger SE, Berninger JP, Carriquiriborde P, Coors A, Deleo PC, Dyer SD, Ericson JF, Gagne F, Giesy JP, Gouin T, Hallström L, Karlsson MV, Larsson DGJ, Lazorchak JM, Mastrocco F, McLaughlin A, McMaster ME, Meyerhoff RD, Moore R, Parrott JL, Snape JR, Murray-Smith R, Servos MR, Sibley PK, Straub JO, Szabo ND, Topp E, Tetreault GR, Trudeau VL, Van Der Kraak G. Pharmaceuticals and personal care products in the environment: what are the big questions? 2012. Environ. Health Perspect. 120(9): 1221-1229.

Carlsson G, Örn S, Larsson DGJ. Effluent from bulk drug production is toxic to aquatic vertebrates. 2009. Environ. Toxicol. Chem. 28(12): 2656-2662.

Fick J, Söderström H, Lindberg RH, Phan C, Tysklind M, Larsson DGJ. Contamination of surface, ground, and drinking water from pharmaceutical production. 2009. Environ. Toxicol. Chem. 28(12): 2522-2527.

Finley RL, Collignon P, Larsson DGJ, McEwen SA, Li XZ, Gaze WH, Reid-Smith R, Timinouni M, Graham DW, Topp E. The scourge of antibiotic resistance: the important role of the environment. 2013. Clin. Infect. Dis. 57(5): 704-710.

Flach CF, Boulund F, Kristiansson E, Larsson DGJ. Functional verification of computationally predicted qnr genes. 2013. Ann Clin Microbiol Antimicrob 12. doi:10.1186/1476-0711-12-34.

Flach CF, Johnning A, Nilsson I, Smalla K, Kristiansson E, Larsson DGJ. Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. 2015. J. Antimicrob. Chemother. 70(10): 2709-2717.

Gaze W, Krone S, Larsson DGJ, Li X-Z, Robinson J, Simonet P, Smalla K, Timinouni M, Topp E, Wellington E, Wright G, Zhu Y-G. Influence of humans on the evolution and mobilization of the environmental antibiotic resistome. 2013. Emerg. Inf. Dis. 19. doi:10.3201/eid1907.120871.

Graham DW, Collignon P, Davies J, Larsson DGJ, Snape J. Underappreciated Role of Regionally Poor Water Quality on Globally Increasing Antibiotic Resistance. 2014. Environ. Sci. Technol. 48(20): 11746-11747.

Gunnarsson L, Kristiansson E, Rutgersson C, Sturve J, Fick J, Forlin L, Larsson DG. Pharmaceutical industry effluent diluted 1:500 affects global gene expression, cytochrome P450 1A activity, and plasma phosphate in fish. 2009. Environ. Toxicol. Chem. 28(12): 2639-2647.

Janzon A, Kristiansson E, Larsson DGJ. Environmental microbial communities living under very high antibiotic selection pressure. Invited book chapter in: Antimicrobial Resistance in the Environment. 2012. Wiley & Blackwell. 483-501.

Johnning A, Kristiansson E, Angelin M, Marathe N, Shouche YS, Johansson A, Larsson DGJ. Quinolone resistance mutations in the faecal microbiota of Swedish travellers to India. 2015. BMC Microbiol 15. doi:10.1186/s12866-015-0574-6.

Johnning A, Moore ER, Svensson-Stadler L, Shouche YS, Larsson DGJ, Kristiansson E. Acquired genetic mechanisms of a multiresistant bacterium isolated from a treatment plant receiving wastewater from antibiotic production. 2013. Appl. Environ. Microbiol. 79(23): 7256-7263.

Jonsson V, Österlund T, Nerman O, Kristiansson E. Statistical evaluation of methods for identification of differentially abundant genes in comparative metagenomics. 2016. BMC Genomics 17. doi:10.1186/s12864-016-2386-y.

Jutkina J, Rutgersson C, Flach CF, Larsson DGJ. An assay for determining minimal concentrations of antibiotics that drive horizontal transfer of resistance. 2016. Sci. Total Environ. 548-549: 131-138.

Kristiansson E, Fick J, Janzon A, Grabic R, Rutgersson C, Weijdegård B, Söderström H, Larsson DGJ. Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. 2011. PloS One, 6. doi:10.1371/journal.pone.0017038.

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

Larsson DGJ. Release of active pharmaceutical ingredients from manufacturing sites--need for new management strategies. 2010. Integr. Environ. Assess. Manag. 6(1): 184-186.
Larsson DGJ. Pollution from drug manufacturing: review and perspectives. 2014. Phil. Trans. Royal Soc. London B: Biol. Sci. 369. doi:10.1098/rstb.2013.0571.

Larsson DGJ, de Pedro C, Paxéus N. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. 2007. J. Haz. Mat. 148(3): 751-755.

Larsson DGJ, Fick J. Transparency throughout the production chain--a way to reduce pollution from the manufacturing of pharmaceuticals? 2009. Regul. Toxicol. Pharmacol. 53(3): 161-163.

Larsson DGJ, Lööf L. Läkemedel i miljön. In: Läkemedelsboken 2014, edited and produced by the Swedish Medical Products Agency. 2014. Pp 1267-1279. Also available at www.lakemedelsboken.se.

Lundström SV, Östman M, Bengtsson-Palme J, Rutgersson C, Thoudal M, Sircar T, Blanck H, Eriksson KM, Tysklind M, Flach CF, Larsson DGJ. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. 2016. Sci. Total Environ. 553: 587-595.

Marathe NP, Regina VR, Walujkar SA, Charan SS, Moore ERB, Larsson DGJ, Shouche YS. A treatment plant receiving waste water from multiple bulk drug manufacturers is a reservoir for highly multi-drug resistant integron-bearing bacteria. 2013. Plos One, 8. doi:10.1371/journal.pone.0077310.

NLS. Nationell läkemedelsstrategi (National pharmaceutical strategy). Swedish Government. https://lakemedelsverket.se/upload/om-lakemedelsverket/NLS/Strategi-%20och%20handlingsplan%20NLS%202016.pdf
PHA. Public health agency of Sweden. Swedish work on containment of antibiotic resistance. https://www.folkhalsomyndigheten.se/pagefiles/17350/Svenskt-arbete-mot-antibiotikaresistens.pdf

Pruden A, Larsson DGJ, Amezquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JM, Suzuki S, Silley P, Snape JR, Topp E, Zhang T, Zhu YG. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. 2013. Environ. Health Persp. 121(8): 878-885.

Rutgersson C, Fick J, Marathe N, Kristiansson E, Janzon A, Angelin M, Johansson A, Shouche Y, Flach CF, Larsson DG. Fluoroquinolones and qnr genes in sediment, water, soil, and human fecal flora in an environment polluted by manufacturing discharges. 2014. Environ. Sci. Technol. 48(14): 7825-7832.

Rutgersson C, Gunnarsson L, Fick J, Kristiansson E, Larsson DG. Oral exposure to industrial effluent with exceptionally high levels of drugs does not indicate acute toxic effects in rats. 2013. Environ. Toxicol. Chem. 32(3): 577-584.

Sandegren L, Salin K, Larsson DGJ. The environmental dimension of antibiotic resistance. In: Uppsala Health Summit – a world without antibiotics. Post-conference report. Pp 17-21.
SOU. Statens offentliga utredningar SOU 2013:23. Swedish Government. Available at http://www.regeringen.se/rattsdokument/statens-offentliga-utredningar/2013/04/sou-201323/

Ågerstrand M, Berg C, Björlenius B, Breitholtz M, Brunström B, Fick J, Gunnarsson L, Larsson DGJ, Sumpter JP, Tysklind M, Rudén C. Improving environmental risk assessment of human pharmaceuticals. 2015. Environ. Sci. Technol. 49(9): 5336-5345.

Evaluate removal of high risk APIs through wastewater treatment

Berndt Björlenius
KTH Royal Institute of Technology

Professor Magnus Breitholtz
Stockholm University

Introduction

Concern of the fate of biological active pharmaceuticals in the environment has increased during the last decades. In a MistraPharma sampling campaign, pharmaceutical residues were found in coastal water around large cities. One compound, carbamazepine, was found in every sample even in distant open sea areas. The pharmaceutical residues in the Baltic Sea have been discharged from wastewater treatment plants or directly from sewer overflows. Today's wastewater treatment plants (WWTPs) remove 40-60% of the active pharmaceutical ingredient (API), excluding bulk APIs paracetamol and ibuprofen, which are removed by 95%. Some of the APIs in the influent to a wastewater treatment plant will accumulate in sludge, but more than 95% will appear in the water phase, why the treatment development focuses on wastewater not sludge.

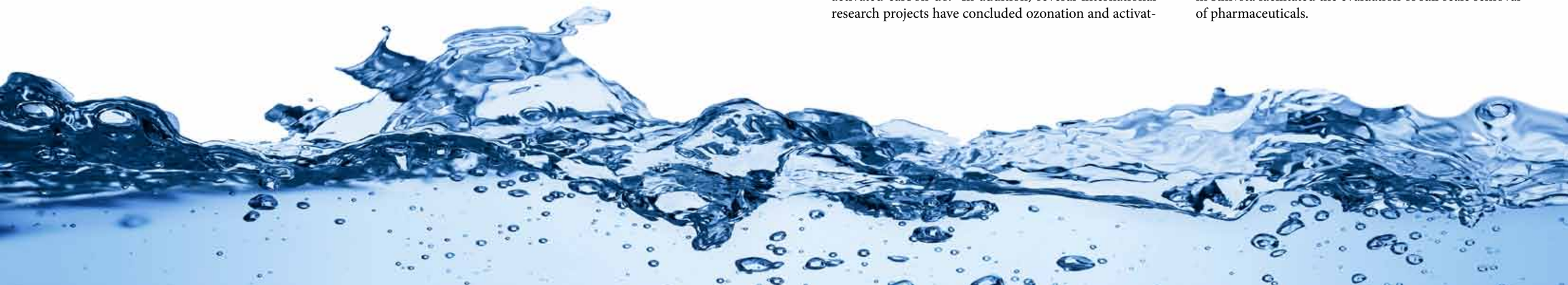
Development of "green" medicines that decompose into inactive and benign substances will take decades, if ever feasible. It would take even longer to replace existing pharmaceuticals. We still have to produce and use our medicines and the residuals will, after passage in our bodies, enter the sewer network or on-site treatment facilities for single households.

End of pipe treatment will therefore be the solution for decades if we want to remove APIs from the water cycle. Upstream control is still very important to prevent unused APIs to be wasted to the sewers. Hospitals, though not contributing with more than a few percent of API to the total amount in a city, are point sources of some APIs, e.g. from clinics where antibiotics are used.

We have divided the feasible treatment technologies for removal of APIs into biological, oxidative and physical methods. Stockholm Water led a project where treatment methods were screened on typical Swedish wastewater treatment facilities. The results pointed out the oxidative method ozonation and the physical method activated carbon as being the two major candidates for future studies. A moving bed biofilm reactor (MBBR) was selected as a reference process from the third screening group: the biological treatment, although this group does not reach the high removal rate as ozonation and activated carbon do. In addition, several international research projects have concluded ozonation and activated carbon as being the most promising technologies also for implementation in full scale.

In the first half of MistraPharma program, 2008-2011, LTH worked extensively with biofilm processes, sludge and activated carbon adsorption and oxidation processes like ozonation and chlorine dioxide.The second half of MistraPharma program, 2012-2015, KTH worked with the selected treatment technologies: ozonation, activated carbon and MBBR.

The overall aim for 2012-2015 was to evaluate processes for removal of pharmaceuticals in parallel, on real wastewater, at three different waste water treatment plants (WWTPs) in a mobile pilot plant. An additional aim was to design, test and evaluate full scale components for removal of pharmaceuticals in municipal wastewater. During the project, a mobile pilot plant for removal of pharmaceuticals was designed, operated and evaluated at four different WWTPs. At the last site, Knivsta WWTP, the pilot plant was operated in parallel with Sweden´s first full scale plant, that we designed within MistraPharma. The cooperation with the full scale plant project in Knivsta facilitated the evaluation of full scale removal of pharmaceuticals.



> *In conclusion the aims for removal of pharmaceutical residues have been reached: Design, operation and evaluation of a mobile plant have been done as well as implementation and tests with full scale components. Being the last link in the chain, scientific papers are still under production during spring 2016.*
>
> Berndt Björlenius

Project progression overview

In May 2012, when KTH joined the project, planning and literature studies for a mobile pilot plant started. During the autumn, design and procurements for the mobile plant started, as well as planning of a diploma work on powdered activated carbon (PAC).

An introductive diploma work on ozonation (O3) and filtration on granular activated carbon (GAC) had already started in January 2012 at Stockholm Water, waiting for the funds to be granted. Lab plants for the diploma work were designed to be operated in parallel. Three granular carbons were evaluated in downstream filters in parallel with test variants of ozonation; bubble diffuser and multi stage ozonation.

Based on the diploma work, literature surveys and in-house experience, a selection and detailed design of pilot plants were performed during the autumn 2012. Plants for ozonation and activated carbon plants, in two major forms were designed: GAC and PAC. Three separate lines were built of both GAC and PAC, to facilitate comparisons of different activated carbon products. In addition, two plants with biofilm-based treatment MBBR were designed to make possible the evaluation of the results from MistraPharma phase 1.

In total, eleven different lines were designed and blueprinted for local production in the workshops at Käppala WWTP and external blacksmiths. The welding of the reactors started in late 2012. Skilled installers and high quality material were used and so the plant has long-term durability.

In parallel with the design of treatment processes, a 6 m (20 ft) shipping container was ordered, insulated and prepared to house the pilot plants. Three 5 m high ozone columns were prepared. Procurements of feed pumps, sampling pumps and on-line meters were performed during late 2012. In 2013,

the mobile lab was completed and taken in operation. All eleven lines worked properly from the start, but some improvements were gradually done, mainly on the PAC-lines, but also of the programming of the control system.

The mobile lab consisted of eleven treatment lines; three containing GAC, another three with PAC, two lines with ozonation, two lines with biofilm (MBBR) and finally one line with sand-filtration (SF) after ozonation. Each GAC-line consists of two downstream filters in series.In the default configuration, one GAC, PAC and MBBR line follows in parallel one ozonation line. By some simple rearrangements the reverse combination is possible: ozonation after PAC or GAC treatment.

An applied control system serves the lines to control inlet pumps, water levels in GAC and sand filters, ozonation dose, dosing of PAC and of flocculation agent. The control system also stores process data for later evaluation. A separate system for data sampling of water flows through the pilot lines and temperatures in the pilot lines, but also for the fish tanks, has been installed.

Ten 50 L fish tanks have been installed in a rack for intermittent bio tests. The tanks are fed with the same wastewater, two and two, so duplicate tests of each sample stream can easily be achieved. Effluent wastewater from the WWTP normally serves as the positive control and tap water as the negative control. The retention time for the wastewater is one hour in each tank. Aeration via ceramic air stones supply the water with sufficient (>95%) oxygen saturation.

A diploma work on PAC addition was performed 2013 on lab scale facilities. The diploma work was carried out at Käppala WWTP. The lab tests, with different PAC-products, at six different doses for each product showed a broad span of removal efficiencies. Based on the lab tests, four products, from two manufacturers were purchased for the pilot tests.

We initiated the sampling campaign of sea water performed yearly since 2013 by the crew on the ship "The Brig Tre Kronor af Stockholm", which is operated by the "Initiative Sustainable Seas". Understanding 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 and not to be released in the environment.

After commissioning of the pilot plant, evaluation tests with flow proportional water sampling started in October 2013 at Käppala WWTP. Weekly samples were collected from 14 sampling points. In addition to the mainly continuous operation of the lines in the pilot plant, factorial experiments with e.g. dose, temperature and pH were performed from time to time.

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 for 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 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 eleven lines. In the end of September 2014 the pilot plant was disassembled again and moved to the 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. On December 15, 2014, the pilot plant was moved back to Käppala WWTP. There complementary tests were done on pre-treatment, control of ozone addition, GAC and PAC.

Table 1. Treatment plants visited and operating time of pilot plant

Sewage treatment plant	Number of weeks
Käppala I:	20 weeks
Uppsala:	10 weeks
Västerås:	8 weeks
Käppala II:	19 weeks
Knivsta:	6 weeks
Total	**63 weeks**

In late 2014, we designed Sweden´s first full scale treatment for removal of micro-pollutants, including pharmaceutical residues. Ozonation was the treatment technology of choice, due to high removal of pharmaceuticals, short construction time, low investment cost and low operating cost. The ozonation step has capacity to treat wastewater from all 10 000 person in the municipality of Knivsta in the Stockholm Region. Knivsta WWTP was chosen due to its reasonable size, 10 000 inhabitants, and the small receiving watercourse, that is heavily influenced by the treated wastewater. The ozonation in Knivsta WWTP constitutes the final treatment step after the existing mechanical, biological and chemical wastewater treatment.

The pharmaceutical removal step is an up scaled plant based on the mobile pilot tests within MistraPharma. In 2015, we led the procurements, construction work, commissioning and operation of the full scale ozonation. The Swedish Agency for Marine and Water Management, SwAM, funded a major part of the project in Knivsta.

In 2015, complementary pilot tests were performed at Käppala and Knivsta WWTPs on pre-treatment ahead of the pilot plants and process performance. The administration systems of ozone into wastewater were redesigned and parts of the pilot plant were reconstructed. The lines with GAC and PAC were in operation for determination of adsorption capacity but also for determination of other design parameters like hydraulic retention time. In total, 63 weeks of pilot plant operation, at four WWTPs were performed (Table 1).

> *”In total 63 weeks of pilot plant operation, at four WWTPs were performed.*
>
> Berndt Björlenius

© Klas Öster

Treatment results

During the tests, different doses, retentions times, pH etc were varied. In tuned tests, PAC and GAC systems showed the highest removal of pharmaceuticals, 95-98%. The corresponding dose or consumption of activated carbon has been in the range of 15-70 g prod/m3. In PAC systems, the activated carbon consumption is typically one half to one third compared to GAC systems. This is due to diffusion limitations and less area displayed in GAC filters. Memantine and venlafaxine had the lowest adsorption on activated carbon.

With an appropriate ozone dose of 5-7 g O3/m3, ozonation reach 85-95%, with lower biomarker responses than today´s effluent. No difference in the performance was recorded between the two ozonation lines. The sand filter treatment after ozonation did not improve the removal of pharmaceuticals. Fluconazol was the most resistant API to ozonation. The full scale ozonation in Knivsta reached the project´s goal of 90% removal of APIs in the effluent from the existing treatment plant.After a long start up time, MBBR eventually reached 20-30% removal. Venlafaxine was among the hardest APIs to remove for the biological treatment.

Ecotoxicity

To evaluate the treatment, chemical analysis of pharmaceuticals is crucial. However, to assay the risk of by-product generation, especially by ozonation, the sensitivity of biological tests is needed. The biotests, with a selection of biomarkers, offer a good indication of the presence of harmful substances in wastewater. To this end, biomarkers in rainbow trout and *Daphnia magna* were analysed. In addition, a standard growth inhibition test with microalgae was used to compare raw and treated wastewater.

The biotests were performed by Uppsala University and ACES at Stockholm University in cooperation with KTH, who designed the fish tanks and built the support systems with feed pumps, heaters, coolers and aeration. In total, three exposure tests with rainbow trout were perform by Uppsala University, one in lab scale in Käppala, one in pilot scale in Käppala and one in pilot scale in Uppsala. Biotests on *Daphnia magna* and microalgae were run at Stockholm University on the same wastewater samples, collected in parallel with the rainbow trout exposure.

The first major rainbow trout exposure was performed at Käppala WWTP during one week in the end of November 2013. Three extensively treated effluent streams (ozonated, ozonated plus sand filtered and GAC-filtered) were compared with the negative control (tap water) and the positive control(effluent from the treatment plant).

The second major rainbow trout exposure was performed at Kungsängsverket in Uppsala during one week in the beginning of September 2014. Three extensively treated effluent streams (sand filtered, ozonated and GAC- filtered) were compared with the negative control (tap water) and the positive control (effluent from the treatment plant).The results from rainbow trout exposures showed that both GAC filtration and ozonation efficiently removed most of the APIs analyzed, and reduced biomarker responses. Ozonation of the water did not result in any increased oxidative stress response in the fish.

A main objective with the biotests on microalgae and *Daphnia magna* was to study potential negative effects due to the formation of by-products, especially by ozonation. So far, results are available for growth inhibition in microalgae exposed to raw wastewater and wastewater treated with O3, O3 + SF, PAC and GAC in pilot scale in Käppala, Uppsala and Västerås. All the biotests on oxidative stress response in *Daphnia magna* have recently been finalized, but the data analysis is yet to be done.

In microalgal tests, the raw wastewater from Västerås and Käppala were found to be toxic, whereas the wastewater from Uppsala had stimulating effects, which makes the comparison of the three WWTP plants somewhat difficult. Still, the results showed that ozonation caused no or positive effects compared to raw wastewater on growth in microalgae, indicating that this additional treatment option at the chosen concentration did not generate toxic by-products for algae. For sand-filtration after ozonation and activated carbon, the effects are different depending on the WWTP location. In Uppsala, sand-filtration caused neither positive nor negative effects, whereas activated carbon overall caused negative effects on algal growth. Since the raw wastewater from Uppsala had

stimulating effects, the latter result probably reflects that essential trace elements were removed rather than that the wastewater became more toxic after the additional treatment. In Västerås, sand-filtration caused marginally negative effects on growth in microalgae whereas in Käppala significant positive effect was observed. Overall, treatment with activated carbon caused either no or slight positive effects compared to raw wastewater at both Västerås and Käppala.

To conclude, none of the additional treatment options applied to wastewater from Västerås and Käppala was sufficiently effective to completely remove chemical agents causing growth inhibition on microalgae. At the same time, no additional negative effects compared to raw wastewater were observed for ozonation. In Uppsala, where raw wastewater had stimulating effects, ozonation in fact had a positive effect on microalgal growth.

Knowledge transfer and impact

Information to all types of stakeholders about applicable technologies and their efficiency for removal of pharmaceutical residues in municipal wastewater has broadened the general knowledge level in the field. Tests with the same pilot plant on four different treatment plants, on real wastewater and with comparable high removal rates (>90%), indicates the general validity of these competitive processes for removal of pharmaceutical residues in municipal wastewater.

We believe that the knowledge from MistraPharma, communicated via meetings with stakeholders, national seminars, reports and the web, has encouraged other actors to form their own evaluation or research projects. Together with funding from The Swedish Agency for Marine and Water Management, SwAM, at least five constellations work with pharmaceutical residues in municipal wastewater in Sweden today.

MistraPharma´s presentation of typical design values and characteristics for ozonation and activated carbon processes has narrowed the value interval for design parameters under Swedish conditions. Implementation of the first Swedish full scale plant for removal of pharmaceutical residues in municipal wastewater is a result of the work within MistraPharma. The work including lab, pilot and full scale operation, evaluation and up scaling was successful. We consider that this progress is one of the "ice breaking" actions that will led to implementation of pharmaceutical treatment steps at several wastewater treatment plants in Sweden, certainly in the long term, but in some cases in the short term.

During the four project years, over 20 persons have been engaged in the work with the research on wastewater treatment and especially on the construction of the pilot and full scale plants: two project leaders, four operators, two diploma workers, two PhD students, seven blacksmiths and three electricians.

Key publications

Björlenius B, Furuhagen S, Gorokhova E, Ek K, Liewenborg B, Breitholtz M. Evaluation of technologies for removal of pharmaceuticals in municipal wastewater – comparison of process data, chemical analysis and biomarkers responses in microalgae and Daphnia magna. *Manuscript in preparation.*

Björlenius B., Kårelid V. Larsson G., "Long term performance of purification techniques of pharmaceutical residues in wastewater". *Manuscript in preparation.*

Björlenius B., Larsson G., "Ozonation in pilot and full scale with pre- and post treatment". *Manuscript in preparation.*

Björlenius B., Tysklind M., Fick J. "Pharmaceutical residues in the Baltic Sea – Occurrence and an example of a substance flow analysis ". *Manuscript in preparation.*

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.

Kårelid V., Larsson G., Björlenius B. "Pilot-scale removal of pharmaceuticals in municipal wastewater; a comparison of granular and powdered activated carbon treatment in three wastewater treatment plants in Sweden". *Manuscript in preparation.*

Kårelid V., Larsson G., Björlenius B. "Removal of pharmaceuticals in municipal wastewater with powdered activated carbon; process optimization in bench and pilot scale". *Manuscript in preparation.*

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

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

McGivney E, Carlsson M, Gustafsson JP, Gorokhova E (2015). Effects of UV-C and Vacuum-UV TiO2 Advanced Oxidation Processes on the acute mortality of microalgae. Photochemistry and Photobiology 91 (5): 1142-1149.

Addressing major issues associated with the environmental risk assessment and management of pharmaceuticals

Doctor Marlene Ågerstrand
Stockholm University

Professor John Sumpter
Brunel University

This chapter presents results from two projects aiming at improved risk assessment and management of pharmaceuticals. First, a new method for reporting and evaluating ecotoxicity studies has been developed. Use of this method contributes towards increased inclusion of peer-reviewed studies in regulatory decision-making. To ensure the method's usability, accuracy and acceptance, it was developed in an international ring test with a wide range of stakeholder.

Second, since pharmaceuticals are present simultaneously in the aquatic environment, it is reasonable to consider them together when conducting a risk assessment. However, knowledge about mixture effects for pharmaceuticals was missing. To advance this area, a number of mixture toxicity experiments on fish were performed. The results show that mixture effects do occur, suggesting that this should be considered when performing risk assessment.

New reporting and evaluation method for peer-reviewed ecotoxicity studies

In order to strengthen the scientific basis of regulatory decisions, new methods that facilitate the use of peer-reviewed studies in hazard and risk assessments of pharmaceuticals, and other chemicals, are needed (Ågerstrand, Breitholtz, and Rudén 2011). In addition, in order for these methods to gain acceptance among risk assessors and researchers on an international arena, a close dialog with end-users is required when developing new tools.

Table 1: Examples of reliability and relevance criteria from the CRED evaluation method. For further explanation of each criterion, and the complete list of criteria, see Moermond et al. 2015.

Reliability criteria (examples)
Are appropriate controls performed (e.g. solvent control, negative and positive control)?
Is the test substance identified with name or CAS-number? Are test results reported for the appropriate compound?
Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?
Are chemical analyses adequate to verify concentrations of the substance over the duration of the study?
Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate used for all controls and test concentrations?
Relevance criteria (examples)
Are the reported endpoints appropriate for the regulatory purpose?
Are appropriate life-stages studied?
Are the experimental conditions relevant for the tested species?
Is the exposure duration relevant and appropriate for the studied endpoints and species?
Is the tested exposure scenario relevant for the substance?

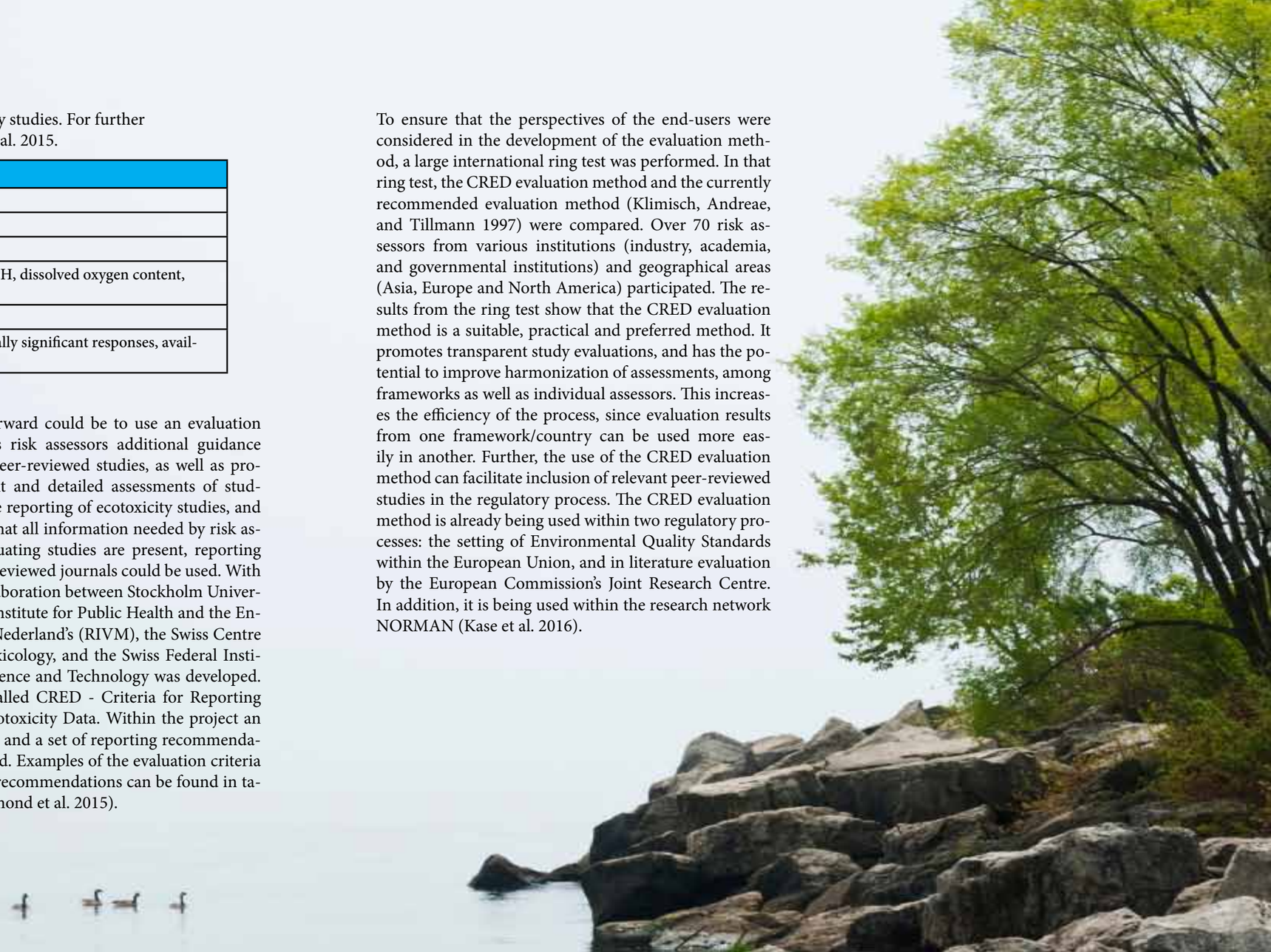
Table 2: Examples of the CRED reporting recommendations for ecotoxicity studies. For further explanation, and the complete list of recommendations, see Moermond et al. 2015.

Reporting recommendations (examples)
Description of controls, control mortality, and validity criteria for the test.
Identification, characteristics, purity and source of the test compound.
Scientific name, age/life-stage, sex, and source of the test organism.
Exposure schedule, test medium composition, feeding protocols, temperature, pH, dissolved oxygen content, description of equipment, preparation of stock solutions.
Nominal and measured concentrations, analytical method, exposure duration.
Number of replicates, statistical method used, dose-response observed, statistically significant responses, availability of raw data.

Regulatory decisions should be based on all available scientific data of sufficient reliability and relevance, this is specified in guidance documents used in chemicals regulation (European Chemicals Agency 2011). However, in practice there is a low use of peer-reviewed (eco)toxicity studies in hazard and risk assessments of chemicals. Instead data from producers and importers of chemicals are primarily used. There are several possible reasons for this gap between chemicals regulation and researchers publishing studies in the peer-reviewed literature. For example, the use of studies performed according to validated guidelines (e.g. from OECD) and Good Laboratory Practices (GLP) are emphasized in available risk assessments guidance documents, time constrains which makes it difficult for risk assessors to search for and evaluate additional studies other than those submitted by the chemical company, and peer-reviewed studies with deficiencies in the design, performance, and/or reporting (Ågerstrand, Edvardsson, and Rudén 2013; Suter II and Cormier 2016). In addition to these factors, there is also a discussion concerning the relevance of the endpoints, test designs and species investigated in ecotoxicity studies (Buonsante et al. 2014).

A possible way forward could be to use an evaluation method that gives risk assessors additional guidance when evaluating peer-reviewed studies, as well as promoting transparent and detailed assessments of studies. To improve the reporting of ecotoxicity studies, and thereby ensuring that all information needed by risk assessors when evaluating studies are present, reporting standards in peer-reviewed journals could be used. With this in mind a collaboration between Stockholm University, the National Institute for Public Health and the Environment in the Nederland's (RIVM), the Swiss Centre for Applied Ecotoxicology, and the Swiss Federal Institute of Aquatic Science and Technology was developed. The project was called CRED - Criteria for Reporting and Evaluating Ecotoxicity Data. Within the project an evaluation method and a set of reporting recommendations was developed. Examples of the evaluation criteria and the reporting recommendations can be found in table 1 and 2 (Moermond et al. 2015).

To ensure that the perspectives of the end-users were considered in the development of the evaluation method, a large international ring test was performed. In that ring test, the CRED evaluation method and the currently recommended evaluation method (Klimisch, Andreae, and Tillmann 1997) were compared. Over 70 risk assessors from various institutions (industry, academia, and governmental institutions) and geographical areas (Asia, Europe and North America) participated. The results from the ring test show that the CRED evaluation method is a suitable, practical and preferred method. It promotes transparent study evaluations, and has the potential to improve harmonization of assessments, among frameworks as well as individual assessors. This increases the efficiency of the process, since evaluation results from one framework/country can be used more easily in another. Further, the use of the CRED evaluation method can facilitate inclusion of relevant peer-reviewed studies in the regulatory process. The CRED evaluation method is already being used within two regulatory processes: the setting of Environmental Quality Standards within the European Union, and in literature evaluation by the European Commission's Joint Research Centre. In addition, it is being used within the research network NORMAN (Kase et al. 2016).



To further help risk assessors structure, share and summarize their evaluations, the SciRAP (Science in Risk Assessment and Policy) color-coding web tool was developed in a collaboration between Stockholm University and Karolinska Institutet. The tool is available free of charge at www.scirap.org. The intention of the tool is to facilitate and increase the use of peer-reviewed (eco)toxicity studies in regulatory assessments, thereby bridging the gap between peer-reviewed research and chemicals regulation (Molander et al. 2014).

The reporting recommendations developed within the CRED-project serve as a template for researchers when designing, performing and reporting ecotoxicity studies. So far, there are no peer-reviewed journals using detailed reporting recommendations for ecotoxicity studies, even though such recommendations have potential to improve the reliability and reproducibility of studies and streamline the publication process by ensuring that essential information is provided. In other research areas, such as epidemiology, in vivo toxicity studies on mammals, and microarray experiments, systematic reporting recommendations have been developed to guide researchers, reviewers and editors in the publication process (Ågerstrand 2016). Currently, several research groups are using the CRED reporting recommendations in their daily work to ensure sufficient reporting of ecotoxicity studies.

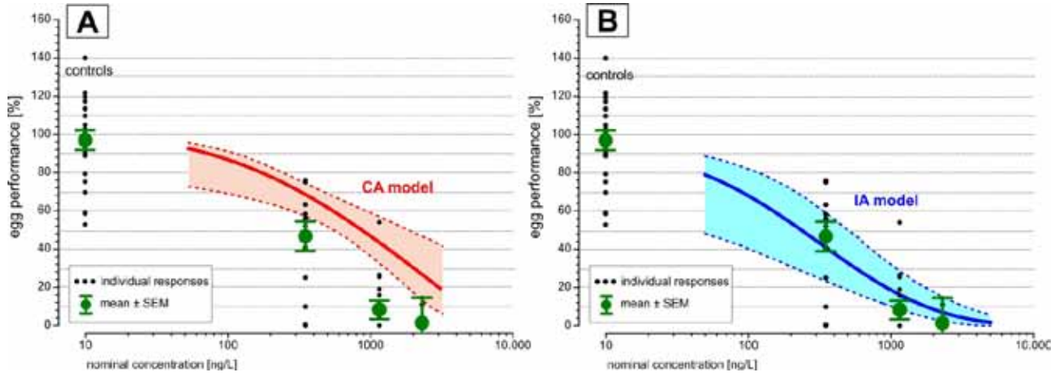


Figure 1: Observed and predicted effects of a mixture of five steroidal pharmaceuticals on the reproductive performance of a model fish species. The concentration of the mixture of pharmaceuticals is shown on the horizontal axis and the number of eggs produced on the vertical axis. 100% represents no change in egg production before and after exposure to the mixture of pharmaceuticals. Data from two experiments have been combined. The actual data are shown in green (mean ± SEM), with the data from each pair of fish represented as individual black dots. The actual data were compared with two predictions, one originating from the model of Concentration Addition (A) and the other the model of Independent Action (B). In both cases the predicted concentration – response relationship ± 95% confidence belt are shown. It is clear that the IA model predicted the reproductive response in experimental fish considerably better than the CA model.

The need to consider mixtures of pharmaceuticals in environmental risk assessment

It is now clear that many pharmaceuticals are present simultaneously in the aquatic environment. Often a number of different representatives of a single class of pharmaceuticals can be detected in a single water sample (Backhaus, 2014). For example, a number of different beta-blockers (e.g. atenolol, bisoprolol, metoprolol, propranolol) are readily detected in effluents from wastewater treatment works and in river water samples. As these pharmaceuticals have the same mechanism of action (MOA), it seems reasonable to consider them together when conducting a risk assessment, rather than conducting a risk assessment on each individual beta-blocker separately. This has been recognised for some time, but how to conduct a risk assessment for a mixture of pharmaceuticals (or for any group of chemicals) is less clear (Kortenkamp et al., 2009). Hence, mixtures toxicity is often incorporated into risk assessment by including an arbitrary safety factor, usually 10, into the assessment. However, if the scientific understanding of mixtures toxicity could be improved, it should be possible to incorporate a more scientifically robust approach into the risk assessment of mixtures of human pharmaceuticals.

Earlier research, conducted before the project began, had demonstrated that if pharmaceuticals had similar MOAs (i.e. all were beta-blockers, or oestrogens), then the response to a mixture could be accurately predicted using additivity (Brian et al., 2005): the model used is termed concentration addition (C.A.). But what would be the response of an aquatic organism, such as a fish, to a mixture of pharmaceuticals with different MOAs? To begin studying this question - which is widely recognised as a very difficult one to answer - fish were exposed to a simple, binary, mixture of a synthetic oestrogen and a synthetic progestogen. Both pharmaceuticals are steroids, and both are used in hormonal contraceptives. Both are also readily detected in wastewater effluents, and hence wild fish are exposed to these pharmaceuticals, albeit at very low concentrations (Zhou et al., 2016). These two pharmaceuticals have different MOAs, yet both affect the same apical endpoint, namely reproduction, although they do so via different physiological mechanisms (Lange et al., 2001; Paulos et al., 2010; Runnalls et al., 2013). It was found that additivity, using the C.A. model, could accurately predict the overall effect of any mixture of these two pharmaceuticals. This result was built on during the project by conducting a series of experiments in which fish were exposed to considerably more complex, and hence environmentally realistic, mixtures of steroid pharmaceuticals. For example, fish were exposed to a mixture containing a synthetic oestrogen, a synthetic androgen, two synthetic progestogens and a synthetic glucocorticoid (the latter pharmaceuticals are anti-inflammatory drugs). After demonstrating that all five pharmaceuticals adversely affected reproduction - albeit via different MOAs - the effects of various mixtures of these pharmaceuticals were assessed. The results very conclusively demonstrated that the response of the fish to the mixture of 5 pharmaceuticals was considerably greater than the response to each of the drugs when they were tested individually; that is, a mixture effect occurred. The C.A. model underestimated the response to these mixtures. In contrast, the model of independent

action (I.A.), which assumes that each pharmaceutical has a different MOA (which was most likely), accurately predicted the effect of the mixture on the reproductive performance of the fish (see Figure 1).

The results of these experiments also demonstrated that 'something from nothing' can occur. This phenomenon occurs when each individual pharmaceutical in the mixture is present at a concentration that is not high enough to produce a measurable effect, but the mixture does produce an effect (Silva et al., 2002).

Overall, the results of a number of experiments demonstrated conclusively that mixture effects do occur when fish are exposed simultaneously to a number of different pharmaceuticals that all, one way or another, affect reproduction adversely. This is perhaps not surprising, but had not been demonstrated before, for any group of pharmaceuticals. It shows that mixtures toxicity should be incorporated into the environmental risk assessment of human pharmaceuticals (Backhaus, 2014). The results also showed that just because an individual pharmaceutical is present in the environment at a concentration below its lowest concentration that causes a measurable effect (the LOEC) does not mean that it poses no harm: it could still contribute to a mixture effect, if other pharmaceuticals affecting the same physiological processes were also present.

Although it is clear that any robust environmental risk assessment of human pharmaceuticals needs to incorporate the possibility of mixtures toxicity, it is less obvious exactly how this can be achieved. In order to be able to conduct such an assessment, it is necessary to know what pharmaceuticals are present in the environment, and at what concentrations. Knowledge of those factors has improved greatly in the last few years, which has been very helpful. Knowledge of the biological effects, and potency, of each pharmaceutical is also required in order to conduct a mixtures risk assessment. This ecotoxicological information is often not available, thus hindering any environmental risk assessment of these pharmaceuticals, either singly or in combination as a mixture. During this project one group of human pharmaceuticals known to adversely affect fish at very low concentrations - the steroidal pharmaceuticals - has been studied both singularly and in various combinations in order to test some of the key principles underlying mixtures toxicity (see also the recent papers by Hua et al., 2016; Zhao et al., 2015). We hope that the results obtained will advance the field, as well as encourage further research on this difficult, but very important, issue, so that in the not-too-distant future mixtures toxicity assessments can be based on sound science, and hence conducted with confidence.

Key publications

Kase R, Moermond C, Korkaric M, Werner I, Ågerstrand M. Criteria for Reporting and Evaluating ecotoxicity Data (CRED): Comparison and perception of the Klimisch and CRED methods for evaluating reliability and relevance of ecotoxicity studies. Accepted for publication in Environmental Science Europe.

Moermond C, Kase R, Korkaric M, Ågerstrand M. 2015. CRED - Criteria for Reporting and Evaluating ecotoxicity Data. Environmental Toxicology and Chemistry, In Press.

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.

Runnalls, T.J., Beresford, N., Kugathas, S., Margiotta-Casaluci, L., Scholze, M., Scott, A.P. and Sumpter, J.P. 2015. From single chemicals to mixtures - reproductive effects of levonorgestrel and ethinylestradiol on the fathead minnow. Aquatic Toxicology 169: 152–167.

Thrupp, T., Runnalls, T.J., Scholze, M., Kortenkamp, A. and Sumpter, J.P. 2016. The effects of mixtures of steroid pharmaceuticals on reproductive capacity of the fathead minnow (Pimephales promelas). Manuscript in preparation.

References

Ågerstrand, Marlene. 2016. In Response: Reporting Recommendations to Ensure Reliability and Reproducibility of Ecotoxicity studies—A Tripartite Initiative. Environmental Toxicology and Chemistry 35 (5): 1072–73.

Ågerstrand, Marlene, Magnus Breitholtz, and Christina Rudén. 2011. Comparison of Four Different Methods for Reliability Evaluation of Ecotoxicity Data: A Case Study of Non-Standard Test Data Used in Environmental Risk Assessments of Pharmaceutical Substances. Environmental Sciences Europe 23 (1): 17.

Ågerstrand, Marlene, Linnéa Edvardsson, and Christina Rudén. 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. Human and Ecological Risk Assessment: An International Journal 20 (6): 1427–45.

Backhaus, t. 2014. Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures. Phil. Trans. Royal Soc. B. 369, 20130885.

Buonsante, Vito A., Hans Muilerman, Tatiana Santos, Claire Robinson, and Anthony C. Tweedale. 2014. Risk Assessment′s Insensitive Toxicity Testing May Cause It to Fail. Environmental Research 135C: 139–47.

Brian, J.V., Harris, C.A., Scholze, M., Backhaus, T., Booy, P., Lamoree, M., Pojana, G., Jonkers, N., Runnalls, T.j., Bonfa, A., Marcomini, A. and Sumpter, J.P. 2005. Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. Environ. Health Perspect. 113, 721-728.

European Chemicals Agency. 2011. REACH Guidance Documents. Helsinki, Finland.

Hua, J., Han, J., Wang, X., Guo, Y and Zhou, B. 2016. The binary mixtures of megestrol acetate and 17 alpha-ethinylestradiol adversely affect zebrafish reproduction. Environ. Poll. 213, 776-784.

Klimisch, H.-J, M Andreae, and U Tillmann. 1997. A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regulatory Toxicology and Pharmacology 25 (1): 1–5.

Kortenkamp, A., Backhaus, T. and Faust, M. 2009. State of the Art Report on Mixture Toxicity. Directorate General for the Environment: Report to the EU Commission.

Lange, R., Hutchinson, T.H., Croudace, C.P., Siegmund, F., Schweinfurth, H., Hampe, P., Panter, G.H. and Sumpter, J.P. 2001. Effects of the synthetic oestrogen 17 alpha-ethinylestradiol over the life-cycle of the fathead minnow (Pimephales promelas). Environ. Toxicol. Chem. 20, 1216-1227.

Paulos, P., Runnalls, T.J., Nallani, G., LaPoint, T., Scott, A.P., Sumpter, J.P. and Huggett, D.B. 2010. Reproductive responses in Fathead Minnow and Japanese Medaka following exposure to a synthetic progestin, Norethindrone. Aquatic Toxicol. 99, 256-262.

Runnalls, T.J., Beresford, N., Losty, E., Scott, A.P. and Sumpter, J.P. 2013. Several synthetic progestins with different potencies adversely affect reproduction of fish. Environ. Sci. Technol. 47, 2077-2084.

Silva, E., Rajapakse, N. and Kortenkamp, A. 2002. Something from "nothing" – Eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. Environ. Sci. Technol. 36, 1751-1756.

Suter II, Glenn W., and Susan M. Cormier. 2016. Bias in the Development of Health and Ecological Assessments and Potential Solutions. Human and Ecological Risk Assessment: An International Journal 22 (1): 99–115.

Zhao, Y.B., Castiglioni, S. and Fent, K. 2015. Environmental progestins medroxyprogesterone acetate and dydrogesterone and their binary mixtures adversely affect reproduction and lead to histological and transcriptional alterations in zebrafish (Danio rerio). Environ. Sci. Tachnol. 49, 4636-4645.

Zhou, L-J., Zhang, B-B., Zhao, Y-G and Wu, Q.L. 2016. Occurrence, spatiotemporal distribution, and ecological risks of steroids in a large shallow Chinese lake, Lake Taihu. Science of the Total Environ. 557-558, 68-79.

Provide state-of-the-art analyses for APIs of environmental concern

Associate professor Jerker Fick
Doctor Richard H. Lindberg
Professor Mats Tysklind
Umeå University

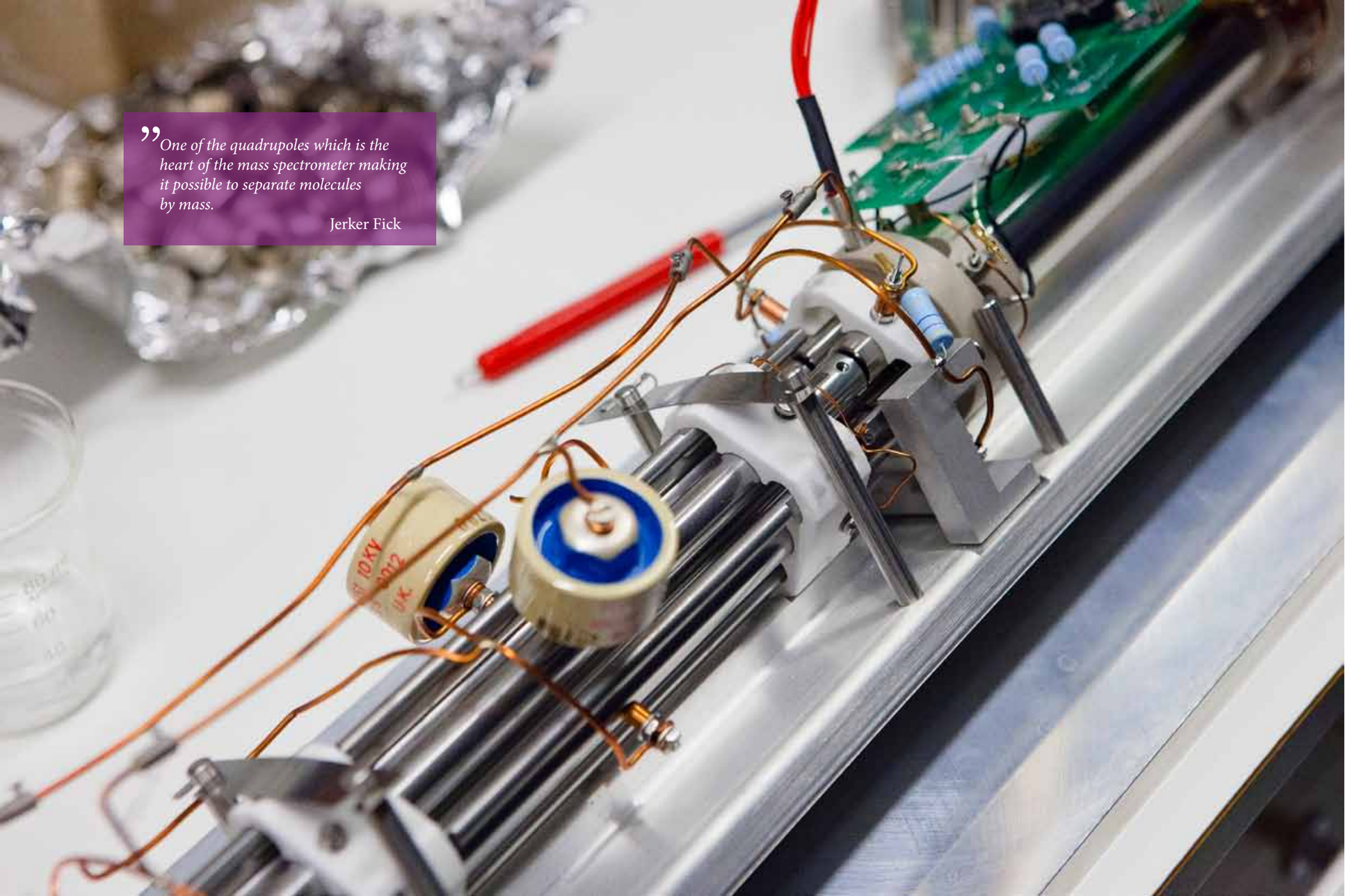
Analysis of pharmaceutical residues in the environment is a rapidly expanding field and analytical methods for additional APIs are published regularly, e.g. Al-Qaim et al., 2014; Aminot et al., 2015; Bayen et al., 2013; Bourdat-Deschamps et al., 2014; Grabic et al., 2012; Huerta et al., 2016; Idder et al., 2013; Lindberg et al., 2014; Petrie et al., 2015. Recent advances in multi-residue analytical methods have made it possible to analyse large number of APIs simultaneously and several publications now include 50+ APIs (Al-Qaim et al., 2014; Aminot et al., 2015; Grabic et al., 2012; Lindberg et al., 2014). These methods are extremely valuable in a screening phase when there is limited information on the occurrence and persistency of investigated APIs. When developing a multi-residue analytical method, great efforts have been made to include as many relevant APIs as possible. In MistraPharma we have used various methods to prioritize APIs but mainly we have based the selection approach on the fish plasma model, first suggested by Huggett et al. (Huggett et al., 2003). This model, described in detail in Chapter 1, is based on the assumption that if two species share the same drug target, the pharmaceuticals are expected to activate this target at roughly the same plasma concentration. In short this approach takes into account sales, potency and physico-chemical properties and provides an estimated risk that exposed biota could have adverse effects. Although the APIs included in the Mistra-Pharma analytical methods were pre-selected using this approach, additional APIs were included in order to test the validity of the selection e.g. by including APIs of as many different classes as possible. One of the major advantages with the fish plasma model is that it enables theoretical risks to be calculated for the great majority of pharmaceuticals since human therapeutic plasma concentrations are readily available in the literature, including APIs that currently lack documented environmental effects or measured concentrations. However, by including APIs with a potential to be of environmental concern a lot of extra pressure is put on the analytical chemistry since the selection is not in any way based on analytical considerations. In addition, as few as possible should be omitted since all of the APIs present on the list are relevant.

The instrumental technique used for analysis of APIs is predominantly based on High Performance Liquid Chromatography - Electrospray Ionization - tandem Mass Spectrometry (HPLC-ESI-MS/MS) (e.g. Al-Qaim et al., 2014; Aminot et al., 2015; Bayen et al., 2013; Bourdat-Deschamps et al., 2014; Grabic et al., 2012; Huerta et al., 2016; Idder et al., 2013; Lindberg et al., 2014; Petrie et al., 2015). In MS/MS, product ions produced by collision of API pre-cursor ions and inert gas are detected, this increases the sensitivity due to a higher selectivity. One of the main challenges in trace analysis is matrix effects; i.e. other chemicals in the sample that either increase or decrease the signal of the APIs of interest (Fatta-Kassinos et al., 2011; Lindberg et al., 2014; Rossmann et al., 2015; Trufelli et al., 2011). This has to be studied for each API and matrix and these effects can be reduced. Of the most common methods employed (each has its limitations), is internal standard (IS) calibration determination, in which each APIs is assigned to a labelled API (or a chemical with close structural similarity to the target APIs), that is added to each sample and all standards. This methodology is relatively cheap, time efficient and corrects for matrix effects to a certain extent. By adding the same amount of each IS to the sample as to the calibration solutions, ratios (based on the peak areas in the chromatograms) of the IS and corresponding analytes are calculated and used for the determination of sample concentrations. Quality assurance is of major concern in the quantification of APIs and has to be emphasized throughout analysis and have been so in the MistraPharma programme. Analytical quality assurance includes e.g. stringent standard controls, careful selection of IS, and investigations of matrix effects. API stability have to be investigated in various matrixes and conditions as well as determinations of APIs and IS extraction yield (recovery) in various aqueous and solid samples.

Within MistraParma a multi-residue method for water samples that simultaneous determine more than 90 APIs, representing 27 distinct classes of pharmaceuticals, was developed and validated (Grabic et al., 2012). The developed method utilizes a single HPLC–MS/MS run after sample enrichment using solid-phase extraction (SPE). No previous method existed for 52 of the 100 target APIs and several analytical parameters were optimized and the final method were validated for surface waters and influent/effluent sewage waters. Absolute recoveries were above 70% for most APIs in most matrices. Limits of quantification (LOQs) ranged from 0.05 to 50ngL-1 (median 5 ngL-1). The use of matrix-matched standards led to a significant reduction matrix effects; i.e. enhancement or suppression of the signals of the APIs. The recoveries of the method for real matrices were in the range of 23–162%.

In order to increase sample throughput an automated method was also developed in MistraPharma (Lindberg et al., 2014). In this study, which included 105 APIs, the targeted APIs were quantified using a multi-residue method based on online SPE-HPLC-MS/MS. This method was thoroughly validated and complies with EU regulations on sample handling, limits of quantification, quality control and selectivity. This method was also thoroughly validated and e.g. matrix effects and filtration recoveries were investigated for each API.

Both of the multi-residue methods have been used to assess levels of selected APIs in various aqueous and solid matrices, for instance: a Swedish screening study conducted during 2010-2011 including sewage waters, sewage sludge, surface waters and biota (Fick et al., 2011); an EU wide sewage effluent investigation including 90 sewage treatment plants (Loos et al., 2013); and several studies regarding optimization of API removal during municipal sewage water treatment (e.g. Björlenius et al., in preparation).



One of the quadrupoles which is the heart of the mass spectrometer making it possible to separate molecules by mass.

Jerker Fick

Although multi-residue methods are important, target analysis of a single APIs or a specific class of APIs is useful. Based on screening results of Swedish waters, a study of the anxiolytic pharmaceutical oxazepam showed that it will alter the behavior of perch at environmentally relevant concentrations (Brodin et al., 2013). Investigations of dose and effect relationships in biota have also been made including e.g. fluoxetine exposure to tadpoles (Berg et al., 2013); bioconcentration and impacts of antihistamines to aquatic insects (Jonsson et al., 2014); and tissue-specific bioconcentration of antidepressants in fish exposed to sewage effluents (Grabicova et al., 2014). Further, Steroid hormones, e.g. estrogenes, progestins, androgens, comprise a group of compounds that have a similar chemical structure as well as phenolic and sometimes aliphatic hydroxyl groups. These groups of APIs have the unfortunate combination of being extremely potent and extremely difficult to analyze. Thus, there is a crucial need of methods for ultra-trace analysis of these compounds and in MistraPharma several specific methods have been developed for the analysis of these compounds, e.g. a method that measure low ng L-1 of the synthetic progestine levonorgestrel was developed (Kvarnryd et al., 2011).

Key publications

Grabic, R., Fick, J., Lindberg, R.H., 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. doi:10.1016/j.talanta.2012.08.032

Kvarnryd, M., Grabic, R., Brandt, I., Berg, C., 2011. Early life progestin exposure causes arrested oocyte development, oviductal agenesis and sterility in adult Xenopus tropicalis frogs. Aquatic Toxicology 103, 18–24. doi:10.1016/j.aquatox.2011.02.003

Lindberg, R.H., 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. Water Research 58, 221–229. doi:10.1016/j.watres.2014.03.076

References

Al-Qaim, F.F., Abdullah, M.P., Othman, M.R., Latip, J., Zakaria, Z., 2014. Multi-residue analytical methodology-based liquid chromatography-time-of-flight-mass spectrometry for the analysis of pharmaceutical residues in surface water and effluents from sewage treatment plants and hospitals. Journal of Chromatography A 1345, 139–153. doi:10.1016/j.chroma.2014.04.025

Aminot, Y., Litrico, X., Chambolle, M., Arnaud, C., Pardon, P., Budzindki, H., 2015. Development and application of a multi-residue method for the determination of 53 pharmaceuticals in water, sediment, and suspended solids using liquid chromatography-tandem mass spectrometry. Analytical and Bioanalytical Chemistry 407, 8585–8604. doi:10.1007/s00216-015-9017-3

Bayen, S., Zhang, H., Desai, M.M., Ooi, S.K., Kelly, B.C., 2013. Occurrence and distribution of pharmaceutically active and endocrine disrupting compounds in Singapore's marine environment: Influence of hydrodynamics and physical-chemical properties. Environ. Pollut. 182, 1–8. doi:10.1016/j.envpol.2013.06.028

Berg C., Backström, T., Winberg, S., Lindberg R., Brandt, I. Developmental Exposure to Fluoxetine Modulates the Serotonin System in Hypothalamus. Plos One, January 28, 2013, DOI: 10.1371/journal.pone.0055053
Björlenius, B., et al. Manuscripts in preparation.

Bourdat-Deschamps, M., Leang, S., Bernet, N., Daudin, J.-J., Nelieu, S., 2014. Multi-residue analysis of pharmaceuticals in aqueous environmental samples by online solid-phase extraction-ultra-high-performance liquid chromatography-tandem mass spectrometry: Optimisation and matrix effects reduction by quick, easy, cheap, effective, rugged and safe extraction. Journal of Chromatography A 1349, 11–23. doi:10.1016/j.chroma.2014.05.006

Brodin, T., Fick, J., Jonsson, M., Klaminder, J. Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations. Science, 15 Feb 2013: Vol. 339, Issue 6121, pp. 814-815. DOI: 10.1126/science.1226850

Fatta-Kassinos, D., Meric, S., Nikolaou, A., 2011. Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research. Anal. Bioanal. Chem. 399, 251–275. doi:10.1007/s00216-010-4300-9

Fick, J., Lindberg, R.H., Kaj, L., Brosström-Lundén, E. Results from the Swedish national screening programme 2010. Subreport 3. Pharmaceuticals. IVL report B2014. 2011.

Grabic, R., Fick, J., Lindberg, R.H., 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. doi:10.1016/j.talanta.2012.08.032

Grabicova, K., Lindberg, R.H., Östman, M., Grabic, R., Randak, T., Larsson, J.D.G., Fick, J. Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. Science of The Total Environment, Volumes 488–489, 1 August 2014, Pages 46–50. http://dx.doi.org/10.1016/j.scitotenv.2014.04.052

Huerta, B., Rodriguez-Mozaz, S., Nannou, C., Nakis, L., Ruhi, A., Acuna, V., Sabater, S., Barcelo, D., 2016. Determination of a broad spectrum of pharmaceuticals and endocrine disruptors in biofilm from a waste water treatment plant-impacted river. Science of the Total Environment 540, 241–249. doi:10.1016/j.scitotenv.2015.05.049

Huggett, D.B., Cook, J.C., Ericson, J.F., Williams, R.T., 2003. A theoretical model for utilizing mammalian pharmacology and safety data to prioritize potential impacts of human pharmaceuticals to fish Human and Ecological Risk Assessment: An International Journal, 9:7, 1789-1799, DOI: 10.1080/714044797.

Idder, S., Ley, L., Mazellier, P., Budzinski, H., 2013. Quantitative on-line preconcentration-liquid chromatography coupled with tandem mass spectrometry method for the determination of pharmaceutical compounds in water. Analytica Chimica Acta 805, 107–115. doi:10.1016/j.aca.2013.10.041

Jonsson, M., Fick, J., Klaminder, j., Brodin, T. Antihistamines and aquatic insects: Bioconcentration and impacts on behavior in damselfly larvae (Zygoptera). Science of The Total Environment, Volume 472, 15 February 2014, Pages 108–111. doi:10.1016/j.scitotenv.2013.10.104

Kvarnryd, M., Grabic, R., Brandt, I., Berg, C., 2011. Early life progestin exposure causes arrested oocyte development, oviductal agenesis and sterility in adult Xenopus tropicalis frogs. Aquatic Toxicology 103, 18–24. doi:10.1016/j.aquatox.2011.02.003

Lindberg, R.H., 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. Water Research 58, 221–229. doi:10.1016/j.watres.2014.03.076

Loos, R., et al. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water Research, Volume 47, Issue 17, 1 November 2013, Pages 6475–6487. http://dx.doi.org/10.1016/j.watres.2013.08.024

Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. Water Research 72, 3–27. doi:10.1016/j.watres.2014.08.053

Rossmann, J., Gurke, R., Renner, L.D., Oertel, R., Kirch, W., 2015. Evaluation of the matrix effect of different sample matrices for 33 pharmaceuticals by post-column infusion. Journal of Chromatography B-Analytical Technologies in the Biomedical and Life Sciences 1000, 84–94. doi:10.1016/j.jchromb.2015.06.019

Trufelli, H., Palma, P., Famiglini, G., Cappiello, A., 2011. An Overview of Matrix Effects in Liquid Chromatography-Mass Spectrometry. Mass Spectrometry Reviews 30, 491–509. doi:10.1002/mas.20298

Programme Communication

Karin Liljelund
Trossa AB

Helene Hagerman
Trossa AB

Marlene Ågerstrand
Stockholm Uiversity

There are several key factors that have been determinative for the successful execution of communicating a complex, multi-stakeholder research program. Here, the most important factors are summarized.

A committed program director

Throughout the program period, MistraPharma's program director has shown great commitment to, and understanding for the need of, communication activities.

An engaged and relevant reference group

The most important stakeholders were involved early on in the MistraPharma program to ensure the commitment and participation of these stakeholders. This gave the communication team the ability to calibrate the objectives and orientation of the communication work according to these stakeholders' interests. Please see next page for more information.

Professional communication agents

Essential for successfully managing outreach in a complex academic topic area was that the communications staff not only have experience from different types of communications, but also knowledge of the topics at hand and an existing professional network in the concerned community including policy makers and researchers. It has been central to continuously analyze and evaluate the need and suitable forms for communication in close collaboration with researchers, the board, reference group and other relevant stakeholders.

Stakeholder communication established as an integrated part of the researchers' work

During the first years, the communication project constituted the hub of both internal and external communication. But as the program proceeded, the researchers integrated communication activities with their own work. These activities included participating in conferences, attending seminars and courses and generally spreading information on research results and gained knowledge, both nationally and internationally. The MistraPharma research program was fortunate to have skillful researchers in communicating their research.

Communication manager co-opted to the board

The communication work has been a top priority for the board and a preceding point on the meeting agenda of the board meetings.

A separate communication budget

Already in the application a separate communication budget was allocated for the entire program period. The dedicated communication budget enabled the necessary long-term strategic planning of communication actions and not having to compete internally over funding was an important success factor.

Interested and participating board members

In addition to the interest and active participation of the board, the board members have had leading positions within their respective organizations with the possibility to influence decision-making processes.

Part of the reference group together with MistraPharma researchers and board - November, 2011

The reference group

A reference group with representatives from the most important stakeholders was established at the start of the program period. Initial a working procedure was developed to ensure interaction and effectiveness for the work with the reference group.

A communication strategy was developed in the initial phase of the research program, defining clear objectives of to whom, how, when and why messages should be communicated. Both the reference group and the board were able to comment on the communication plan. The reference group was also involved in the annual update of the corresponding communications plan.

There was also early on a mapping of the expectations and requirements of the reference group on the programme. The researchers were informed on its contents and the document was updated continuously throughout program. Formulating and using this document was very important as it enabled us to ensure realistic expectations from stakeholders.

The close collaboration with an engaged and competent reference group throughout the program proved to be not only informative and appreciated; it also increased the number of direct contacts trough personal meetings and telephone contacts.

Throughout the program period, meetings with the reference group were held twice per year. One of these meetings were held in connection to the annual two-day program meeting, where all of the researchers and the board met to account for their deliverables and to plan for coming deliverables. These meetings were greatly appreciated by the researchers and the reference group, as it provided a discussion forum for various topics.
The communication team worked alongside the reference group to continuously probe their need of communication support and access suggestions on communication activities.

A number of study visits were arranged together with the reference group, visiting for example waste water treatment plants and Astra Zeneca's research laboratory in

The reference group included representatives from MistraPharma's most important stakeholders.
The following organisations (including authorities and companies) participated:

- Ministry of the Environment
- The Swedish Medical Products Agency
- The Swedish Chemicals Agency
- The Swedish Environmental Protection Agency
- The National Food Administration
- The Swedish agency for marine and water management
- The Swedish Institute for Communicable Disease Control
- The Dental and Pharmaceutical Benefits Agency
- Swedish Association of Local Authorities and Regions
- LIF - the Research-based Pharmaceutical Industry in Sweden
- The Association for Generic Pharmaceuticals (FGL)
- The Association for Generic Pharmaceuticals (FGL)
- The Swedish Water & Wastewater Association (SWWA)
- Gryaab
- Stockholm Water Company
- National Coordinator for Social Responsibility in Public Procurement at SKL
- The Swedish Pharmacy Association
- The Swedish County councils trough Blekinge County Council, Västmanland County Council and Kronoberg County Council
- Uppsala University Hospital
- Stockholm South General Hospital (Södersjukhuset AB)
- The International POPs Elimination Network (IPEN)
- ChemSec
- Water Authority for the Northern Baltic Sea

England.

Communication activities

During the programme a great number of communication activities have been carried out, to a great extent in collaboration with our stakeholders. Here we present a selection of activities.

Book release party

A release party for our first book "A Healthy Future - pharmaceuticals in a sustainable society" produced in collaboration between Apoteket AB and Stockholm County Council) was held at Nalen, with over 100 participants.

Popular Science Books

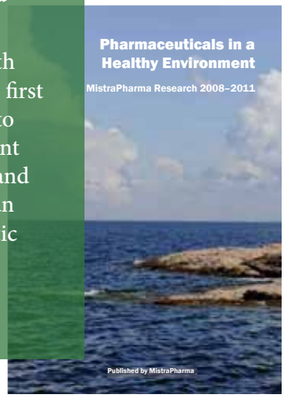
Exemples of the books we produced to highlite MistraPharma research

Collaborating to reduce the environmental risks of pharmaceuticals

This book focused on the importance of collaboration between scientists and stakeholders to achieve common goals. MistraPharma stakeholders presented how they work to identify and reduce the environmental risks of pharmaceuticals, and describe how MistraPharma research benefits their work.

Pharmaceuticals in a Healthy Environment MistraPharma Research 2008—2011

The min purpose of the fourth book was to take stock of the first phase of MistraPharma and to provide an overview of current knowledge, policy activities and future needs regarding human pharmaceuticals in the aquatic environment.



Kick-off

In May, the first year, a kick-off with over 100 participants including our stakeholders (authorities, the pharmaceutical industry, healthcare sector representatives, water treatment sector and pharmacies) was arranged. MistraPharma's projects and objectives were presented and the conference was moderated by the science journalist Charlotte Permell.

Best practice - research communication pamphlet

In 2013, MistraPharma communication team was asked by Mistra to develop a pamphlet on how the research program had designed its communication strategy. The pamphlet, "MistraPharma – focus on communication" was used to showcase a good example of research communication and distributed to the other research programs financed by Mistra.

Seminar "From tablet to toilet"

Together with the Swedish Water & Wastewater Association we arranged this seminar where the main purpose was to highlight concrete actions that different organizations could implement to reduce the load of pharmaceuticals in our waste water system. The seminar's program was designed to mirror chronologically the journey from the manufacture of pharmaceutical products to the release of pharmaceuticals to waste water systems through the toilet. The seminar's "tour guide" was the environmental journalist Charlotte Permell. The following representatives participated:

- The Medical Products Agency,
- The Ministry of Health and Social Affairs,
- The Dental and Pharmaceutical Benefits Agency,
- The research-based pharmaceutical industry association (LIF)
- Blekinge and Stockholm County Council,
- The Pharmaceutical Committee in Västmanland,
- The Swedish Pharmacy Association,
- The Swedish food retail

The seminar was attended by over 110 participants including representatives from MistraPharma's stakeholders.

Almedalen - the politician week

In the summer of 2014 and 2015 MistraPharma arranged seminars during the Almedalen week, Sweden's largest meeting between policy makers, academia, industry and the civil society togheter 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.

In 2014, MistraPharma held the seminar "How do pharmaceuticals affect the Baltic Sea?". The seminar's panel group, including representatives from the Medical Products Agency, the Pharmaceuticals Industry, the Swedish Chemicals Agency, wastewater treatment plants and researchers, discussed which actions were needed and what actions different stakeholders can take to reduce the negative effects of pharmaceutical residues in our water system.

In 2015, MistraPharma arranged a seminar on resistance to antibiotics, titled "Antibiotics – a life and death issue". The seminar was well visited by various decision-makers. Researchers and representatives from the Medical Products Agency, the Pharmaceuticals Industry, the Public Health Agency, the Swedish Water association, healthcare representatives and the national coordinator for socially responsible procurement discussed what is needed from different stakeholders to reduce the spread of resistance to antibiotics and to reduce the number of deaths caused by this development.

Picture (from the left): Lena Ek, Minister for the Environment (2014), Göran Lindstedt, CEO, Sustainable Seas Initiative, Professor Magnus Breitholtz, Stockholm University (MistraPharma) and Tina Elfwing, head of Baltic Sea Center at Stockholm University.

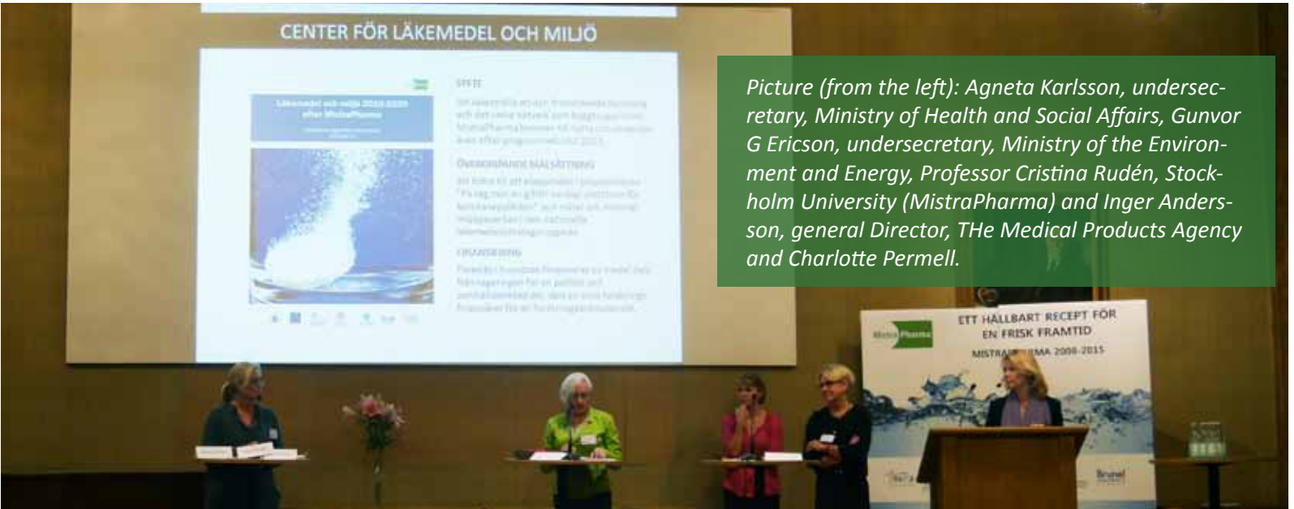
The Brig Tre Kronor af Stockholm - the scene for the seminars

Closing conference - A sustainable recipe for a healthy future

On October 14, 2015, MistraPharma arranged our closing conference "A sustainable recipe for a healthy future". The conference was targeted at stakeholders and researchers and was held at the Medical Society's premises in Stockholm. Approximately 200 people attended in the much appreciated conference.

MistraPharma's project manager presented the main results achieved during the program's eight years. The results were illustrated by, and physically printed on, pieces of a puzzle which is yet to be completed. This symbolized the contributions the research program had made to the research community, while highlighting that the context is still largely unknown and that a lot of research and work remains to be done. The different pieces of the puzzle were handed over to relevant corresponding stakeholders who then commented on how their organizations can learn from and benefit from the results. Each receiving stakeholder organization was represented by its director-general or CEO.

Many discussions were initiated during the day which was finally concluded with MistraPharma's chairman handing over a proposal to establish a Center of Excellence on Pharmaceuticals and Environment to the state secretaries at the Ministry of the Environment and Energy and the Ministry of Social Affairs. The purpose of this proposal, among other things, is to support the interim national target to increase, by 2020, the environmental concern in both the EU's pharmaceutical legislation and internationally in accordance to the government bill "On the way towards a toxic-free environment – platform for the national chemicals policy."



Picture (from the left): Agneta Karlsson, undersecretary, Ministry of Health and Social Affairs, Gunvor G Ericson, undersecretary, Ministry of the Environment and Energy, Professor Cristina Rudén, Stockholm University (MistraPharma) and Inger Andersson, general Director, THe Medical Products Agency and Charlotte Permell.

Media coverage

The researchers in the MistraPharma program participated in a large number of interviews in magazines, newspapers, TV, documentaries/video recordings and radio.

National and international network

In order to reach out to all relevant stakeholders, the program was quick to establish a national and international network. This network was expanded throughout the program period representing different organisations. A newsletter was produced and distributed to the network in a digital format twice a year.

Popular science reports

Yearly reports on the progress made in the MistraPharma research program were produced in order to communicate the research results, but also to highlight their usefulness to different stakeholders.

Student interaction

Throughout the program, the communication team supported students at different academic levels with material and guidance for various theses and dissertation projects.

Main achievements of the MistraPharma program and its communications

Very successful research combined with successful communication activities generated several results, including:

- The creation of a unique network nationally in the field of pharmaceuticals and the environment.
- Highlighting, nationally and internationally, the challenges posed by pharmaceuticals in the environment.
- Contributing to putting Sweden at the forefront of knowledge and research on this topic on the international arena
- Contribution of material to the Swedish Medical Products Agency, enabling them to work more actively on better regulation in the authorization of medicinal products at the EU level.
- Contributing to establishing Pharmaceuticals and Environment as its own field of action in the national pharmaceuticals strategy.
- Contributing to establishing Pharmaceuticals and Environment as its own topic area with detailed objectives in the environmental committee's proposals for a non-toxic environment.
- Contributing to the former Environmental Management Council's environmental criteria for the procurement of pharmaceuticals.
- Providing a knowledge-base for the determination of which pharmaceuticals to include in the Environmental Protection Agency's environmental monitoring.
- Providing a knowledge-base to the new EU water directive regarding the selection of pharmaceutical substances.
- Providing technical advice and decision-making support for our stakeholders.

POLICY BRIEF

Recommendations for Improving Environmental Risk Assessment of Pharmaceuticals

MistraPharma has developed ten recommendations recommendations for improved environmental risk assessment of human pharmaceuticals. These recommendations are based on up-to-date scientific knowledge concerning pharmaceuticals' effects and presence in the environment, as well as experiences from other legal frameworks. To facilitate communication of these recommendations to decision makers we developed a policy brief (see box X). The content of this policy brief has been communicated to decision makers at the European and Swedish Parliaments, as well as to our stakeholder network including personnel from the pharmaceutical industry, the health care sector, and governmental agencies. The policy brief is available at our website.

1 *Require environmental risk assessment also for products put on the market before 2006*

We recommend that environmental risk assessments are performed also on pharmaceutical products approved before the European Medicines Agency's guideline came into force. There is simply no scientific evidence that products put on the market before 2006 are of less environmental concern than new products.

Add requirements to assess the risk for development of antibiotic resistance 2

We recommend that information that enables assessment of the risk for increased antibiotic resistance development is included in the environmental risk assessment for antibiotic substances. This would provide a more accurate picture of the risks associated with environmental occurrence of antibiotics.

3 *Perform only one environmental risk assessment per pharmaceutical substance*

We recommend that pharmaceutical companies that produce or import the same pharmaceutical substances submit a joint environmental risk assessment instead of submitting one per company. This would provide decision makers with coherent information, avoid duplication of work and reduce animal testing.

Refine the tiered approach 4

We recommend that the test approach is refined to include pharmacological and toxicological data from the drug discovery process, as well as bioconcentration data. This would improve the prioritization process for further testing so that ecotoxicity testing is focused on the most problematic substances and the most relevant test organisms.

5 *Perform mixture toxicity assessments on pharmaceutical substance with similar modes of action*

We recommend that environmental risk assessments also consider the total exposure for groups of pharmaceutical substances with similar modes of action. This would enable a more accurate assessment of the environmental risks.

6 *Mandate use of all available ecotoxicity studies*

We recommend that research studies of sufficient reliability and relevance are used in the environmental risk assessment. This would make better use of the available knowledge and could provide decision makers with important informati

7 *Include environmental risks in the risk-benefit analysis*

We recommend that environmental risks are included in the risk-benefit analysis when a product is considered for market authorization. This would make the assessment of risks associated with the use of pharmaceuticals more complete and hence more accurate.

8 *Require review of the environmental risk assessments at regular intervals*

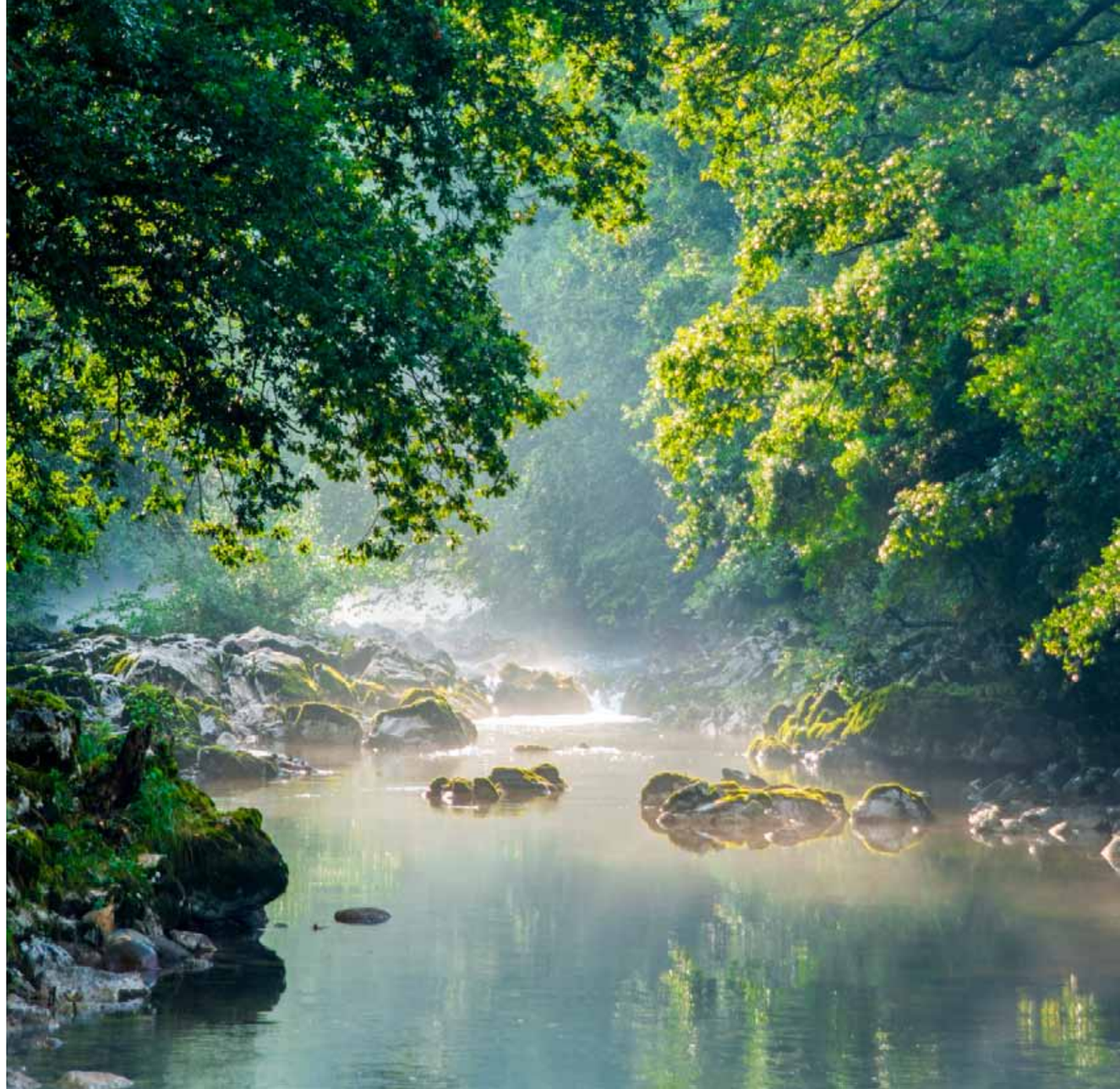
We recommend that environmental risk assessments must be updated when significant new information is available. This would bring forward the regulatory use of new scientific data and may also increase collaboration between stakeholders.

9 *Include data on emissions from production of pharmaceutical substances*

We recommend that the risk associated with discharges from manufacturing sites is included in environmental risk assessments. This would enhance the relevance of the assessments by including the part of the product lifecycle responsible for the highest environmental concentrations detected.

10 *Increase transparency*

We recommend that environmental risk assessments and information about manufacturing sites are made publicly available. This would enable use of that information for other purposes such as research and external evaluation, and it would encourage companies to take more environmental responsibility throughout their supply chains.



THE MISTRAPHARMA TEAM

Marlene Ågerstrand
Doctor, Stockholm University
magnus.breitholtz@aces.su.se

Christina Rudén
Professor, Stockholm University
christina.rudén@aces.su.se

Erik Kristiansson
Associate Professor, Chalmers University of Technology
erik.kristiansson@chalmers.se

Joakim Larsson
Professor, Sahlgrenska Academy, University of Gothenburg.
joakim.larsson@fysiologi.gu.se

Magnus Breitholtz
Professor, Stockholm University
magnus.breitholtz@aces.su.se

Gen Larsson
Professor, KTH Royal Institute of Technology
gen@biotech.kth.se

Björn Brunström
Professor, Uppsala University
bjorn.brunstrom@ebc.uu.se

Berndt Björlenius
KTH Royal Institute of Technology
berndt.bjorlenius@biotech.kth.se

John Sumpter
Professor, Brunel University
John.Sumpter@brunel.ac.uk

Ingvar Brandt
Chair of Environmental Toxicology, Evolutionary Biology Centre, Uppsala Universiy.
ingvar.brandt@ebc.uu.se

Jerker Fick
Associate Professor, Department of Chemistry, Umeå University.
jerker.fick@chem.umu.se

Cecilia Berg
Professor, Uppsala University
bjorn.brunstrom@ebc.uu.se

Hélène Hagerman
Science communicator, Trossa AB
helene.hagerman@trossa.se

Karin Liljelund
Science communicator, Trossa AB
karin.liljelund@trossa.se

Mats Tysklind
Professor in Environmental Chemistry, Umeå University.
mats.tysklind@chem.umu.se

Publications

Stockholm University

Project leader
Marlene Ågerstrand

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.

Project leader
Magnus Breitholtz

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.

Project leader
Mats Tysklind

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.

Bengtsson-Palme J, Flach C-F, Fick J, Kristiansson E, Larsson DGJ. 2014 Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Front. Microbiol. 5:648. doi: 10.3389/fmicb.2014.00648

Brodin T, Piovano S, Fick J, Klaminder J, Heynen M, Jonsson M. 2014 Ecosystem effects of pharmaceuticals in aquatic systems – impacts through behavioural modifications Philosophical Transactions B 369 20130580; doi:10.1098/rstb.2013.0580

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 69(6), 1282-1288.

Grabicova K, Lindberg RH, Östman M, Grabic R, Randak T, Larsson DGJ, Fick J. 2014. Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. Science of the Total Environment 488-489, 46-50.

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.

Klaminder J, Fick J, Jonsson M, Sundelin A, Brodin T. 2014. The conceptual imperfection of aquatic risk assessment tests: highlighting the need for tests designed to detect therapeutic effects of pharmaceutical contaminants Environmental Research Letters 9; 084003

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. Water Research 58, 221-229.

Rutgersson C, Fick J, Marathe N, Kristiansson E, Janzon A, Flach CF, Larsson DGJ. 2014. Fluoroquinolones and qnr genes in sediment, water, soil and human fecal flora in an environment polluted by manufacturing discharges Environmental Science & Technology 48, 7825-7832.

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.

Säfholm M, Jansson E, Ribbenstedt A, Fick J, Berg C. 2014 Risks of hormonally active pharmaceuticals to amphibians: A growing concern regarding progestagens Philosophical Transactions B 369 20130577; doi:10.1098/rstb.2013.0577

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 In Press Aquatic Toxicology

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

Daneshvar A, Prévost M, Fick J, Kronberg L, Weyhenmeyer GA. 2014 Natural waters remove pharmaceuticals faster than nutrients. *Submitted manuscript*

Fick J, Brodin T, M. Heynen, J. Klaminder, M. Jonsson, K. Grabicova, T. Randák, R. Grabic, J. Slobodnik, A. Sweetman, M. Earnshaw, R. Loos 2015 EU-wide monitoring survey of anxiolytics in surface water. Submitted manuscript

Golovko O, Fick J, Lindberg RH, Östman M, Grabic R. 2014. Phototransformation of pharmaceuticals in water under artificial ultraviolet and natural sunlight irradiation. *Submitted manuscript.*

Heynen M, Fick J, , Piovano S, Jonsson M, Klaminder J, Brodin T 2015 Species-specific bioconcentration and biomagnification potential determine realized exposure to pharmaceuticals in aquatic ecosystems. *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.*

Ramstedt M, Hedlund T, Fick J, Björn E, Jahnke I 2015. Re-Thinking Chemistry Education Towards Technology-Enhanced Problem-Based Learning. *Submitted manuscript.*

Ågerstrand M, Berg C, Björlenius B, Breitholtz M, Brunström B, Fick J, Gunnarsson L, Larsson DGJ, Sumpter JP, Tysklind M, Rudén C. 2015. Improving environmental risk assessment of medicinal products for human use. *Submitted manuscript.*

Almroth BC, Fick J, Lindberg RH, Axelsson M, Seth H, Larsson DGJ. 2015 The anti-arrhythmic drug flecainide: environmental detection and probable conserved mode of action in fish. *Manuscript in preparation.*

Björlenius B, Ripszám M, Haglund P, Lindberg RH, Tysklind M, Fick J Pharmaceutical residues are ubiquitous in Baltic Sea coastal and offshore waters. *Manuscript in preparation.*

Gentili F, Fick J. 2015. Algal cultivation on urban wastewater: an efficient way to reduce pharmaceutical and chemicals pollutants. *Manuscript in preparation.*

Klaminder, J. Brodin, T. Sundelin, A. Jonsson, M, Andersson, J. Fick, J. 2015 Decadal long persistence of an anxiolytic drug (oxazepam) in a large freshwater lake. *Manuscript in preparation.*

Publications associated projects

Berglund B, Fick J, Lindgren PE. 2014 Urban wastewater effluent increases antibiotic resistance gene concentrations in a receiving Northern European river. Environmental Toxicology and Chemistry, 9999, 1–5.

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.

Berglund B, Khan GA, Fick J, Lindgren PE. 2014 Abundance and dynamics of antibiotic resistance genes and integrons in lake sediment microcosms PLoS ONE 9(9): e108151. doi:10.1371/journal.pone.0108151

Singer AC, Järhult JD, Grabic R, Khan GA, Lindberg RH, Fedorova G, Fick J, Bowes MJ, Olsen B, Söderström, H. 2014, Intra- and Inter-pandemic Variations of Antiviral, Antibiotics and Decongestants in Wastewater Treatment Plants and Receiving Rivers PLoS ONE 9(9): e108621. doi:10.1371/journal.pone.0108621

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.*

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

J. Fick, T. Brodin, M. Heynen, M. Jonsson, J. Klaminder. Bensodiazepines; bioconcentration and bioaccumulation in various biota and presence i European surface waters. SETAC, Basel, Switzerland, 2014-05-11 to 2014-05-15

J. Fick, B. Björlenius, M. Ripszám, P. Haglund, R.H. Lindberg, M. Tysklind, Pharmaceuticals in the Baltic Sea, NECC, Reykjavik, Iceland, 2014-06-11 to 2014-06-13.

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.

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

Project leader
Joakim Larsson

Published manuscripts

Bengtsson-Palme J, Larsson DGJ. (2016) Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International. 86:140-149. doi:10.1016/j.envint.2015.10.015

J Jonsson V, Österlund T, Nerman O, Kristiansson E, Statistical Evaluation of Methods for Identification of Differentially Abundant Genes in Comparative Metagenomics, BMC Genomics 17(1) 2016.

utkina J, Rutgersson C, Flach CF, Larsson DGJ. (2016) An assay for determining minimal concentrations of antibiotics that drive horizontal transfer of resistance. Science of the Total Environment. 548–549:131–138. doi:10.1016/j.scitotenv.2016.01.044

Lundström SV, Östman M, Bengtsson-Palme J, Rutgersson C, Thoudal M, Sircar T, Blanck H, Eriksson KM, Tysklind M, Flach CF, Larsson DGJ. (2016) Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. Science of the Total Environment. . 553: 587-595.

Bengtsson-Palme J, Hartmann M, Eriksson KM, Pal C, Thorell K, Larsson DGJ, Nilsson RH. (2015) Metaxa2 – Improved Identification and Taxonomic Classification of Small and Large Subunit rRNA in Metagenomic Data, Molecular Ecology Resources, DOI: 10.1111/1755-0998.12399.

Bengtsson-Palme J, Angelin M, Huss M, Kjellqvist S, Kristiansson E, Palmgren H, Larsson DGJ, Johansson A. (2015) The human gut microbiome as a transporter of antibiotic resistance genes between continents. Antimicrobial Agents and Chemotherapy. doi: 10.1128/AAC.00933-15

Boulund F, Sjögren A, Kristiansson E, Tentacle: distributed quantification of genes in metagenomes, GigaScience 4(1) 2015.

Carney Almroth BM, Gunnarsson LM, Cuklev F, Fick J, Kristiansson E, Larsson DGJ. (2015) Waterborne beclomethasone dipropionate affects the physiology of fish while its metabolite beclomethasone is not taken up. Science of the Total Environment 511:37-46. doi:10.1016/j.scitotenv.2014.12.016.

Flach CF, Johnning A, Nilsson I, Smalla K, Kristiansson E, Larsson DGJ. (2015) Isolation of novel IncA/C and IncN fluoroquinolone resistance plasmids from an antibiotic-polluted lake. Journal of Antimicrobial Chemotherapy. 70(10):2709-2717. doi: 10.1093/jac/dkv167

Johnning A, Kristiansson E, Fick J, Weijdegard B, Larsson DGJ. (2015) Resistance mutations in gyrA and parC are common in Escherichia communities of both fluoroquinolone-polluted and uncontaminated aquatic environments. Front. Microbiol. 6:1355. doi: 10.3389/fmicb.2015.01355

Johnning A, Kristiansson E, Martin A, Marathe NP, Shouche YS, Johansson A, Larsson DGJ. (2015) Quinolone resistance mutations in the faecal microbiota of Swedish travellers to India. BMC Microbiology. 15:235. doi:10.1186/s12866-015-0574-6

Wernersson A-S et al (in total 46 authors). (2015) The European technical report on aquatic effect-based monitoring tools under the water framework directive. Environmental Sciences Europe. 27:7. doi:10.1186/s12302-015-0039-4.

Ågerstrand M, Berg C, Björlenius B, Breitholtz M, Brunstrom B, Fick J, Gunnarsson L, Larsson DGJ, Sumpter JP, Tysklind M, and Rudén C. (2015) Improving environmental risk assessment of human pharmaceuticals. Environ. Sci. Technol. 49, 5336–5345. DOI: 10.1021/acs.est.5b00302

Bengtsson-Palme J, Boulund F, Fick J, Kristiansson E, Larsson DGJ. (2014) Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Front. Microbiol. 5:648. doi: 10.3389/fmicb.2014.00648

Bengtsson-Palme J, Boulund F, Fick J, Kristiansson E, Larsson DGJ. (2014) Shotgun metagenomics reveals a wide array of antibiotic resistance genes and mobile elements in a polluted lake in India. Front. Microbiol. 5:648. doi: 10.3389/fmicb.2014.00648.

Grabicova K, Lindberg RH, Östman M, Grabic R, Randak T, Larsson DGJ, Fick J. (2014) Tissue-specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. Science of the Total Environment, 488-489, 46-50. doi:10.1016/j.scitotenv.2014.04.052.

Graham DW, Collignon P, Davies J, Larsson DGJ, Snape J. (2014) Underappreciated role of regionally poor water quality on globally increasing antibiotic resistance. Environmental Science & Technology, vol 48, 11746–11747. http://doi.org/10.1021/es504206x

Kookana RS, Williams M, Boxall AB , Larsson DGJ, Gaw S, Choi K, Yamamoto H, Shashidhar T, Zhu Y-G, Carriquiriborde P. (2014) Potential Ecological Footprints of Active Pharmaceutical Ingredients: An Examination of Risk Factors in Low-, Middle-, and High-income Countries. Philosophical Transactions of the Royal Society B, 369: 20130586. http://dx.doi.org/10.1098/rstb.2013.0586

Larsson DGJ. (2014) Antibiotics and antibiotic resistance in the external environment (in Swedish). Chapter in a popular science book on Antibiotic resistance (Antibiotika - boten och hoten) within the publication series "Formas fokuserar". ISBN 978-91-540-6082-5. Pp 63-72
Larsson DGJ. (2014) Antibiotics in the environment. Upsala Journal of Medical Sciences, 119(2), 108-112 doi:10.3109/03009734.2014.896438

Larsson DGJ and Greko C. (2014) Great drugs in the wrong place: risks for environmental effects and resistance promotion (in Swedish). Läkartidningen no 14, vol 111, pp 619-620. Online copy available here.

Larsson DGJ and Lööf L. (2014) Läkemedel i miljön. In: Läkemedelsboken 2014, edited and produced by the Swedish Medical Products Agency. Pp 1267-1279. Also available at www.lakemedelsboken.se

Rutgersson C, Fick J, Marathe N, Kristiansson E, Janzon A, Angelin M, Johansson A, Shouche Y, Flach CF, Larsson DGJ. (2014) Fluoroquinolones and qnr genes in sediment, water, soil and human fecal flora in an environment polluted by manufacturing discharges. Environmental Science & Technology 48(14)):7825–32. http://dx.doi.org/10.1021/es501452a

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.

Beijer K, Gao K, Jönsson M, Larsson DGJ, Brunström B, Brandt I. 2013. Effluent from drug manufacturing affects cytochrome P450 1 regulation and function in fish. Chemosphere 90: 1149–1157. doi: 10.1016/j.chemosphere.2012.09.023.

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.

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.

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

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.

Pruden A, Larsson DGJ, Amézquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JR, Suzuki S, Silley P, Snape JR, Topp E, Zhang T, Zhu Y-G. (2013) Management Options For Reducing The Release Of Antibiotics And Antibiotic Resistance Genes To The Environment. Environmental Health

Perspectives. vol 121, pp 878-885. doi: 10.1289/ehp.1206446.

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, 13:695, 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.

Svahn S, Göransson U, El-Seedi H, Bohlin L, Larsson DGJ, Olsen B, Chryssanthou E. (2012) Antimicrobial activity of filamentous fungi isolated from highly antibiotic-contaminated river sediment. Infection Ecology and Epidemiology. Vol 2: 11591 - http://dx.doi.org/10.3402/iee.v2i0.11591

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.

Reviews, reports and book chapters

Bengtsson-Palme J, Larsson DGJ. 2015. Antibiotic resistance genes in the environment: prioritizing risks. Nature Reviews Microbiology. 13, 396. doi:10.1038/nrmicro3399-c-1

Graham DW, Collignon P, Davies J, Larsson DGJ, Snape J. 2014. Underappreciated role of regionally poor water quality on globally increasing antibiotic resistance. Environmental Science & Technology, vol 48, 11746–11747. http://doi.org/10.1021/es504206x

Larsson DGJ and Sandegren L. (2015) The environmental dimension of antibiotic resistance. In: Uppsala Health Summit – a world without antibiotics. Pre-conference report. Pp 16-19. www.uppsalahealthsummit.se.

Sandegren L, Salin K, Larsson DGJ. (2015) The environmental dimension of antibiotic resistance. In: Uppsala Health Summit – a world without antibiotics. Post-conference report. Pp 17-21. www.uppsalahealthsummit.se.

Larsson DGJ. 2014. Pollution from drug manufacturing: review and perspectives. Philosophical Transactions of the Royal Society B, 369: 20130571. http://dx.doi.org/10.1098/rstb.2013.0571

Kookana RS, Williams M, Boxall AB , Larsson DGJ, Gaw S, Choi K, Yamamoto H, Shashidhar T, Zhu Y-G, Carriquiriborde P. 2014. Potential Ecological Footprints of Active Pharmaceutical Ingredients: An Examination of Risk Factors in Low-, Middle-, and High-income Countries. Philosophical Transactions of the Royal Society B, 369: 20130586. http://dx.doi.org/10.1098/rstb.2013.0586.

Larsson DGJ. 2014. Antibiotics and antibiotic resistance in the external environment (in Swedish). Chapter in a popular science book on Antibiotic resistance (Antibiotika - boten och hoten) within the publication series "Formas fokuserar". ISBN 978-91-540-6082-5. Pp 63-72.

Larsson DGJ. 2014. Antibiotics in the environment. Upsala Journal of Medical Sciences, 119(2), 108-112 doi:10.3109/03009734.2014.896438.

Larsson DGJ and Greko C. 2014. Great drugs in the wrong place: risks for environmental effects and resistance promotion (in Swedish). Läkartidningen no 14, vol 111, pp 619-620. Online copy available here.

Larsson DGJ and Lööf L. 2014. Läkemedel i miljön. In: Läkemedelsboken 2014, edited and produced by the Swedish Medical Products Agency. Pp 1267-1279. Also available at www.lakemedelsboken.se

Larsson DGJ and Lööf L. 2014. Läkemedel i miljön. In: Läkemedelsboken 2014, edited and produced by the Swedish Medical Products Agency. Pp 1267-1279. Also available at www.lakemedelsboken.se

Ashbolt NJ, Amézquita A, Backhaus T, Borriello SP, Brandt KK, Collignon P, Coors A, Finley R, Gaze WH, Heberer T, Lawrence J, Larsson DGJ, McEwen SA, Ryan JJ, Schönfeld J, Silley P, Snape JR, van den Eede C, Topp E. 2013. Human health risk assessment (HHRA) for environmental development and transfer of antibiotic resistance. Environmental Health Perspectives 121:993-1001. DOI:10.1289/ehp.1206316

Pruden A, Larsson DGJ, Amézquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JR, Suzuki S, Silley P, Snape JR, Topp E, Zhang T, Zhu Y-G. 2013. Management Options For Reducing The Release Of Antibiotics And Antibiotic Resistance Genes To The Environment. Environmental Health Perspectives. vol 121, pp 878-885. doi: 10.1289/ehp.1206446. Chinese translation published in Journal of Occupational Medicine and Health, 2014, vol 31, pp72-77.

Finley RL, Collignon P, Larsson DGJ, McEwen SA, Li X-Z, Gaze WH, Wright GD, Reid-Smith R, Timinouni M, Ryan JJ, Woodland C, Graham DW, Topp E. (2013) The scourge of antibiotic resistance: the important role of the environment. Clinical Infectious Diseases. 57(5):704-710. doi:10.1093/cid/cit355

Gaze WH, Krone SM, Larsson DGJ, Li XZ, Robinson JA, Simonet P, Smalla K, Timinouni M, Topp E, Wellington EM, Wright GD, Zhu YG. 2013. Influence of humans on evolution and mobilization of environmental antibiotic resistome. Emerging Infectious Diseases. Vol 19 (7) Online report: http://dx.doi.org/10.3201/eid1907.120871. DOI:10.3201/eid1907.120871

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

Brandt I, Breitholtz M, la Cour Jansen J, Liljelund K, Larsson DGJ, Rudén C, Tysklind M. (2012) Pharmaceuticals in a Healthy Environment. ISBN: 978-91-978836-2-7. Elanders, Sverige AB Stockholm. 71pp.

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

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

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

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

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.

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.

Selected Popular communication

Fagerberg B, Larsson DGJ, Hagström B. 2012. Prispressade läkemedel utan miljöhänsyn kan ståoss 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" betweenduring 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 UNIVERSITET

Project leader
Ingvar Brandt

Published manuscripts

Gao K, Yan P, Tan CL, Luo YH, Sun J, Jönsson ME, Brandt I, Tang YP. Chinese Journal of Environmental Science (Huan Jing Ke Xue). 2015 Oct;36(10):3878-83. In Chinese.

Jansson E, Mattsson A, Goldstone J, Berg C. 2016. Sex-dependent expression of anti-Müllerian hormone (amh) and amh receptor 2 during sex organ differentiation and characterization of Müllerian duct development in Xenopus tropicalis. General and Comparative Endocrinology, in press.

Säfholm M, Jansson E, Fick J, Berg C. 2016. Molecular and histological endpoints for developmenta reproductive toxicity in Xenopus tropicalis: Levonorgestrel perturbs anti-Müllerian hormone and progesterone receptor expression. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 181–182: 9–18.

Ågerstrand M, Berg C, Björlenius B, Breitholtz M, Brunström B, Fick J, Gunnarsson L, Larsson DG, Sumpter JP, Tysklind M, Rudén C. 2015. Improving environmental risk assessment of human pharmaceuticals. Environmental Science and Technology 49(9):5336-45.

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 161: 146–153.

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.

Gao K, Brandt I, Goldstone J V, Jönsson M E. Cytochrome P450 1A, 1B, and 1C mRNA induction patterns in three-spined stickleback exposed to a transient and a persistent inducer. Comparative Biochemistry and Physiology - Part C. 2011;154(1):42-55.

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

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

Jönsson ME, Gao K, Olsson JA, Goldstone JV, Brandt I: Induction patterns of new CYP1A genes in environmentally exposed rainbow trout. Aquatic Toxicol., 2010, 98, 311-121.

Berg C, Gyllenhammar I., Kvarnryd M. 2009. Xenopus tropicalis as a Test System for Developmental and Reproductive Toxicity. Journal of Toxicology and Environmental Health, 72:219-225.

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

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

Manuscripts in preparation/submitted

Svensson, J., Mustafa, A., Fick, J., Schmitz, M., Brunström, B. (2016) Developmental exposure to progestins causes male bias and precocious puberty in zebrafish (Danio rerio). Submitted.

Svensson, J., Mentor, A., Fick, J., Brunström, B. (2016) Androgenic and anti-estrogenic potencies of progestins and other environmental androgens in female three-spined stickleback (Gasterosteus aculeatus). Manuscript

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.

Berg C. 2012. An Amphibian Model for Developmental and Reproductive Toxicity. Methods in Molecular Biology 889: 73-83.

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

Participated in the cinema documentary film ”Submission” (Underkastelsen) concerning chemical safety in the society, by Stefan Jarl. 2010.

Tidningen Apoteket. No 3, 2009. Läkemedel i miljön. Apoteket AB.

Forskning och Framsteg No 4. 2007. ”Utsläpp får grodor att byta kön

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

Johan Svensson; Progestagenic Aquatic Contaminants Act as Potent Androgens in Fish – experimental studies in three-spined stickleback and zebrafish. Doctoral Thesis, Uppsala University, Sweden. Manuscript. Completed in April 2016.

Kristina Beijer: Azoles and contaminants in Treated Effluents Interact with CYP1A and CYP19 in Fish. Defended 4 June 2015.

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.

Irina Gyllenhammar: Endocrine Disruption in Amphibians. Developmental Effects of Ethinylestradiol and Clotrimazole on the Reproductive System. Defended 28 September 2008.

Licentiate Thesis

Jansson E. 2015. Licentiate thesis. Ontogenetic Characterization of Müllerian Duct development and Expression of anti- Müllerian hormone and other Genes, Uppsala University

Kvarnryd M. 2011. Licentiate thesis. The frog as a model for studies on reproductive toxicity of progestagenic environmental pollutants, Uppsala University.

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

Master of Science/Bachelor theses

Larsson E. 2015. Effects of the hormonally active pesticide propiconazole on ovary and brain aromatase activity in female Xenopus tropicalis. MSc thesis. Master Programme in Environmental Toxicology, Uppsala University.

Eriksson A. 2015. Developmental reproductive effects of the anti-androgenic pesticide linuron on female Xenopus tropicalis. MSc thesis. 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 thesis no. 14:049, Master Programme in Environmental Toxicology, Uppsala University.

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

Jindrisek N. 2013. Exponeringsförsök på yngel av Xenopus tropicalis med miljörelevanta progesteronkoncentrationer - Effekter på amh, ipr och mpr beta. BSc project report. Uppsala University.

Mathieu, N. 2012. Impact of norethindrone and progesterone on gonadal aromatase activity in Xenopus tropicalis. BSc project report. Uppsala University.

Eriksson, A. 2012. Effects of levonorgestrel exposure on spermatogenesis in adult male frogs Xenopus tropicalis. BSc report no 151. Ecotoxicological Education, Uppsala University.

Norder, A. 2011. Progestin exposure impacts oogenesis in adult Xenopus tropicalis frogs. Report no. 143, Ecotoxicological Education, Uppsala University.

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

Eriksson H. 2010. Effekter av etinylöstradiol på aromatasaktiviteten i gonader och hjärna hos Xenopus tropicalis under könsdifferentieringen. MSc thesis no 137. Ecotoxicological Education, Uppsala University.

Söderqvist, A. 2008. Effekter fluoxetin på metamorfos och könsdifferentiering hos Xenopus tropicalis. MSc program in Aquatic and Environmental Engineering, Uppsala University.

Kvarnryd, M. 2008. Effekter av clotrimazol på könsdifferentiering och aromatasaktivitet i gonader och hjärna hos Xenopus tropicalis. MSc thesis no. 124 Ecotoxicological Education.

Conference presentations

Berg C. Risks of progestogens to amphibians: ng/L-concentrations inhibit egg development. SETAC (Society of Environmental Toxicology and Chemistry), Barcelona, Spain. 2015. Oral presentation.

Säfholm M, Jansson E, Fick J, Berg C. Effects on mRNA expression of progesterone receptors (pgrs) after early life exposure to the environmental progestagen levonorgestrel. PRIMO 18, Trondheim, Norge, 2015. Poster.

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 anti-androgens 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

Project leader
Gen Larsson

Manuscripts in preparation/submitted

Flyborg L., Björlenius B., Ullner M. and Persson "A PLS model for predicting rejection of trace organic compounds by nano filtration using treated wastewater as feed"

Björlenius B, Flyborg L, Larsson G. "Comparison of mainstream and sidestream ozonation of municipal wastewater for removal of pharmaceutical residues – ozonation in pilot scale of effluent wastewater and retentate from nano filtration and reverse osmosis"

Björlenius B., Carlsson A., Flyborg L. and Paxeus N. "Removal of pharmaceuticals in municipal wastewater by biological oxidation, chemical oxidation and physical processes: A broad screening study"

Kårelid V., Larsson G., Björlenius B. "Pilot-scale removal of pharmaceuticals in municipal wastewater; a comparison of granular and powdered activated carbon treatment in three wastewater treatment plants in Sweden"

Björlenius B., Tysklind M., Fick J. "Pharmaceutical residues in the Baltic Sea – Occurrence and an example of a substance flow analysis "

Kårelid V., Larsson G., Björlenius B. "Removal of pharmaceuticals in municipal wastewater with powdered activated carbon; process optimization in bench and pilot scale"

Björlenius B., Larsson G., "Ozonation in pilot and full scale with pre- and post treatment" Björlenius B., Kårelid V. Larsson G., "Long term performance of purification techniques of pharmaceutical residues in wastewater"

Björlenius B., Furuhagen S., Gorokhova E., Ek K., Liewenborg B., Breitholz M. " Evaluation of technologies for removal of pharmaceuticals in municipal wastewater – comparison of process data , chemical analysis and biomarker responses in algae, Daphnia Magna and Rainbow trout"

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 LONDON

Project leader
John Sumpter

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'.

MistraPharma worked to identify human pharmaceuticals that are likely to be of concern to aquatic ecosystems and addressed the risk for antibiotic resistance promotion in the environment. MistraPharma also proposed risk management strategies, in particular improved regulatory test requirements and wastewater treatment technologies.

MistraPharma was funded by the Swedish Foundation for Strategic Environmental Research (Mistra) and was ongoing between 2008 and 2015.

www.mistrapharma.se

MISTRA
Stiftelsen för miljöstrategisk forskning

ROSSA GUIDES FOR GOOD
UPPSALA UNIVERSITET
UMEÅ UNIVERSITET
Brunel UNIVERSITY WEST LONDON
Stockholms universitet
KTH ROYAL INSTITUTE OF TECHNOLOGY
GÖTEBORGS UNIVERSITET