Pharmaceuticals – improved removal from municipal wastewater and their occurrence in the Baltic Sea

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Till min älskade familj
Abstract

Pharmaceutical residues are found in the environment due to extensive use in human and veterinary medicine. The active pharmaceutical ingredients (APIs) have a potential impact in non-target organisms. Municipal wastewater treatment plants (WWTPs) are not designed to remove APIs.

In this thesis, two related matters are addressed 1) evaluation of advanced treatment to remove APIs from municipal wastewater and 2) the prevalence and degradation of APIs in the Baltic Sea.

A stationary pilot plant with nanofiltration (NF) and a mobile pilot plant with activated carbon and ozonation were designed to study the removal of APIs at four WWTPs. By NF, removal reached 90%, but the retentate needed further treatment. A predictive model of the rejection of APIs by NF was developed based on the variables: polarizability, globularity, ratio hydrophobic to polar water accessible surface and charge. The pilot plants with granular and powdered activated carbon (GAC) and (PAC) removed more than 95% of the APIs. Screening of activated carbon products was essential, because of a broad variation in adsorption capacity. Recirculation of PAC or longer contact time, increased the removal of APIs. Ozonation with 5-7 g/m^3 ozone resulted in 87-95% removal of APIs. Elevated activity and transcription of biomarkers indicated presence of xenobiotics in regular effluent. Chemical analysis of APIs, together with analysis of biomarkers, were valuable and showed that GAC-filtration and ozonation can be implemented to remove APIs in WWTPs, with decreased biomarker responses.

Sampling of the Baltic Sea showed presence of APIs in 41 out of 43 locations. A developed grey box model predicted concentration and half-life of carbamazepine in the Baltic Sea to be 1.8 ng/L and 1300 d respectively.

In conclusion, APIs were removed to 95% by GAC or PAC treatment. The additional treatment resulted in lower biomarker responses than today and some APIs were shown to be widespread in the aquatic environment.

Keywords

Advanced wastewater treatment, WWTP, pilot plant, pharmaceutical residues, removal of pharmaceuticals, activated carbon, ozonation, nanofiltration, biomarker, Baltic Sea
Sammanfattning


I avhandlingen diskuteras två forskningsområden 1) utvärdering av avancerad rening för att ta bort läkemedelsrester från kommunalt avloppsvatten och 2) förekomst och nedbrytning av läkemedelsrester i Östersjön.

En stationär och en mobil pilotanläggning med nanofiltrering (NF), aktiverat kol och ozonering designades för att studera avskiljning av läkemedelsrester vid fyra reningsverk. NF avskilde 90% av dessa, men retentatet måste behandlas ytterligare. En väl predikterande modell för avskiljningen med NF utvecklades med variablerna: polariserbarhet, sfäriskhet, kvoten mellan hydrofob och polär tillgänglig yta och ämnets laddning. Linjerna med granulerat (GAC) och pulveriserat (PAC) aktiverat kol tog bort minst 95% av läkemedelsresterna. Urvalstest av aktiverat kol är viktiga pga stor variation i adsorptionskapacitet mellan olika produkter. Recirkulation av doserad PAC eller längre kontakttid ökade avskiljningsgraden. Ozonering med dosen 5–7 g/m³ gav 87–95% avskiljning av läkemedelsrester. Biomarkörer i regnbågslax indikerade förekomst av xenobiotika i dagens utgående vatten. Den avancerade rening som utvecklats, minskade signifikant biomarkörernas respons. Kemisk analys i kombination med analys av biomarkörer visade att ozonering och aktiverat kol kan användas i reningsverk för att ta bort läkemedelsrester till 90%-95%.

En provtagning av Östersjön visade på förekomst av läkemedelsrester på 41 av 43 platser. En utvecklad ”grey-box” modell predikterade koncentration och halveringstid av karbamatizepin i Östersjön till 1,8 ng/l respektive 1300 d.

Sammanfattningsvis visade studien att läkemedelsrester kunde avskiljas till 95% med aktiverat kol, med lägre respons i biomarkörer än idag och att de är vitt spridda i miljön.

Nyckelord
Avancerad avloppsvattenrenning, reningsverk, pilotanläggning, läkemedel, avskiljning av läkemedelsrester, aktiverat kol, ozonering, nanofiltrering, biomarkör, Östersjön
List of publications

This thesis is based on the following publications:

Paper I.


Paper II.


Paper III.


Paper IV.


Paper V.


¹Equal contribution.
**Contribution to appended publications**

**Paper I:** BB initiated the study and planned the major sampling campaign, collected additional data, developed and evaluated the model for predicting environmental concentrations. He wrote major parts of the manuscript and was the corresponding author.

**Paper II:** BB initiated the study, proposed the strategy of using MVA, designed the pilot setup and took part in the design of the experiments, operation of the pilot plant and in the evaluation of data. BB derived the final equation for prediction of rejection of substances by nanofiltration. He wrote equal parts of the manuscript.

**Paper III.** BB initiated the study, designed the pilot plants, wrote parts of the manuscript and planned and performed the experiments together with Victor Kårelid (VK). BB planned, evaluated and made parts of the bench scale screening of PAC products. BB was the principal supervisor of the PhD student VK.

**Paper IV.** BB initiated the study, design the flexible PAC lines, wrote parts of the manuscript and was responsible for the original treatment concept. He was together with VK responsible for the planning and execution of the experiments. BB was the principal supervisor of the PhD student VK.

**Paper V.** BB initiated the study, designed the pilot plants and fish tank system, operated the pilot plant during the Käppala biotests and evaluated the chemical analysis. He wrote parts of the manuscript and was the corresponding author.
Related publications not included in this thesis


**Reports**


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<thead>
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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AC</td>
<td>Activated carbon</td>
</tr>
<tr>
<td>AGS</td>
<td>Aerated granular sludge</td>
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<tr>
<td>AMR</td>
<td>Antimicrobial resistance</td>
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<td>AOP</td>
<td>Advanced oxidation processes</td>
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<td>API</td>
<td>Active pharmaceutical ingredient</td>
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<tr>
<td>BNR</td>
<td>Biological nitrogen removal</td>
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<tr>
<td>BOD</td>
<td>Biochemical oxygen demand</td>
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<tr>
<td>BV</td>
<td>Bed volume</td>
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<tr>
<td>CAS</td>
<td>Conventional activated sludge</td>
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<tr>
<td>CEC</td>
<td>Critical environmental concentration</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
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<tr>
<td>CUR</td>
<td>Carbon usage rate</td>
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<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
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<tr>
<td>DDD</td>
<td>Daily defined dose</td>
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<tr>
<td>DOC</td>
<td>Dissolved organic carbon</td>
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<tr>
<td>EBCT</td>
<td>Empty bed contact time</td>
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<tr>
<td>EQS</td>
<td>Environmental Quality Standard</td>
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<tr>
<td>ERA</td>
<td>Environmental risk assessment</td>
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<tr>
<td>EROD</td>
<td>Ethoxyresorufin-O-deethylase</td>
</tr>
<tr>
<td>GAC</td>
<td>Granular activated carbon</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
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<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
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<tr>
<td>MEC</td>
<td>Measured environmental concentration</td>
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<tr>
<td>MLSS</td>
<td>Mixed liquor suspended solids</td>
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<tr>
<td>MWCO</td>
<td>Molecular weight cut off</td>
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<tr>
<td>NF</td>
<td>Nanofiltration</td>
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<tr>
<td>NSAIDS</td>
<td>Non-steroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>OSSF</td>
<td>On-Site Sewage Facilities</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>PAA</td>
<td>Peracetic acid</td>
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<tr>
<td>PAC</td>
<td>Powdered activated carbon</td>
</tr>
<tr>
<td>PCA</td>
<td>Principle Components Analysis</td>
</tr>
<tr>
<td>PE</td>
<td>Population equivalents</td>
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<tr>
<td>PEC</td>
<td>Predicted environmental concentration</td>
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<tr>
<td>PLS</td>
<td>Partial Least Squares Projection of Latent Structures Analysis</td>
</tr>
<tr>
<td>RO</td>
<td>Reverse osmosis</td>
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<tr>
<td>SS</td>
<td>Suspended solids</td>
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<tr>
<td>STP</td>
<td>Sewage treatment plant</td>
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<tr>
<td>TOC</td>
<td>Total organic carbon</td>
</tr>
<tr>
<td>VRF</td>
<td>Volume reduction factor</td>
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<tr>
<td>WFD</td>
<td>Water Framework Directive</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WWTP</td>
<td>Wastewater treatment plant</td>
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Ideas are like rabbits. You get a couple and learn how to handle them, and pretty soon you have a dozen.

*John Steinbeck*
1 Introduction to pharmaceuticals in wastewater

Residues of active pharmaceutical ingredients (APIs) are found in the environment due to extensive use in human and veterinary medicine\textsuperscript{1–5}. The APIs are designed to pass the stomach intact and fit in molecular receptors in humans which are often evolutionary conserved in a variety of organisms leading to a potential impact of APIs in non-target organisms in the environment\textsuperscript{6–8}. The human body conjugates many of the APIs to facilitate the excretion of APIs via urine\textsuperscript{9}. Most of the APIs consumed by humans are excreted in urine and feces, which to a large extent are collected by sewer systems. Some APIs are used externally on skin and will also mainly end up in the sewer system after shower and bath. The existing municipal wastewater treatment plants (WWTPs) are not designed to remove pharmaceutical residues and the most advanced WWTPs remove up to a maximum average of 50-60\% of the pharmaceuticals in the influent\textsuperscript{1,10}.

The present work focused on treatment of effluent from municipal WWTPs since 95\% of the APIs will appear in the water phase and only a small minority of API will accumulate in sludge. Traditional upstream control measures are valuable but will only influence 5-10\% of the number of pharmaceuticals in the wastewater – in many cases we still must take our medicines and the residuals will, after passage in our bodies, enter the sewer network or on-site sewage facilities for single households. Examples of upstream control is to lower the doctors’ prescription of antibiotics or prescription of physical activity instead of medication. A potential upstream measure is the development of “green” medicines that decompose into non-persistent, non-toxic or not bio accumulative substances, but they will take decades to bring to the market, if ever feasible, to replace existing pharmaceuticals. Hospitals, though not contributing with more than a few percent of API to the total amount in a city\textsuperscript{10}, are point sources of some APIs e.g. from clinics where antibiotics are used and are potential target for implementation of pretreatment before discharge to WWTPs. Several technologies have been proposed to remove APIs in municipal wastewater and the most promising can also be implemented in pretreatment facilities at hospitals, but only after a pretreatment which also must be installed to degrade bulk organic substances in hospital wastewater.

In summary, 90-95\% of the mass of APIs in municipal wastewater has passed human bodies, which suggests that end of pipe treatment at municipal
WWTPs must be implemented, if we want to remove APIs from the water cycle. The end of pipe installations will be the main solution for decades, but upstream control is important until biologically degradable APIs are brought to the market.

1.1 The history of water and wastewater treatment and management

Water and wastewater management has a long history. Irrigation of agricultural land was the earliest application of water management. The long-term increasing population in cities has demanded solutions for water supply and wastewater disposal and the policy of all times has been development and improvement of the water and wastewater systems.

1.1.1 Ancient history

Irrigation of farmland was the first organized water management measure taken starting before urban development of water distribution and sewer networks. In Egypt, dams were constructed to level out flooding and facilitate storage of water for irrigation. Examples of early urban water and wastewater application come from Habuba Kebira, a Sumerian settlement, where terracotta pipes were used for urban drainage and water distribution in 3500 BC. The layout of the city drainage system in Habuba Kebira was however not a first-time design, but incorporated knowledge of already existing systems in the Sumerian metropolis of Uruk, situated 900 km from the settlement\textsuperscript{11,12}. Toilet facilities from different eras in Mesopotamia were found in a few houses of the Akkadian (2335–2155 BC) and at Tell Asmar, but also toilet structures from earlier eras have been found\textsuperscript{13}. In 3500 to 3000 BC, many cities in Mesopotamia had networks of wastewater and stormwater drainage. The Indus civilization had bathrooms in houses and sewers in streets 3000 BC\textsuperscript{14}. During early civilizations, the water distribution in urban locations included canals transferring water from rivers, rainwater harvesting systems, wells, aqueducts, and underground storage tanks\textsuperscript{15}.

The Minoans and Mycenaeans built aqueducts from springs connected with tunnels and self-cleaning pipes. The water distribution system included sedimentation basins for removal of silt. During the later Hellenistic period, pressurized pipes and inverted siphons were incorporated in water distribution systems. The cisterns for drinking water, built in many places, were also a safety in wartime. In the Minoan era, lavatories were flushed with reused grey water or stormwater and this principle remained in use in later eras\textsuperscript{14}. 


1.1.2 Water and wastewater management in Rome and the Roman empire

The history of water management in Rome starts 600 BC with the first stretch of Cloaca Maxima. It was first built during the eras of Etruscan kings as drainage from an area in Rome, that later would be Forum Roman, to the river Tiber. The channel had stone walls with wooden bridges that also prevented the walls from collapsing\(^\text{16}\). Side channels from public lavatories “latrina” and street run-off were later connected to Cloaca Maxima, which then was converted to be a combined sewer tunnel by a construction of a roof on top of the stone walls. No channels or pipes from private buildings were connected. The houses were connected to cesspits instead\(^\text{17}\). At least twelve other major combined sewers (cloaca) were built in Rome before 1 BC\(^\text{18}\).

The first centuries after the foundation of Rome in 753 BC, water from wells and river Tiber was used as water supply. Rome had later its major water supply by the aqueducts of which the oldest Aqua Appia, built 312 BC, was 16 km long, stretching mainly underground and had a discharge capacity of 73 000 m\(^3\) per day. The last constructed aqueduct suppling Rome with water, Aqua Alexandrina was built in 226 AD. At the end of the Republic, the one million inhabitants in Rome benefited from a daily supply of some 453 000 m\(^3\) water\(^\text{19}\). Frontinus, senator and responsible water commissioner or director for the advanced water supply, named as “curator aquarum” for the city of Rome, was in charge 97-103 AD. He described in a booklet, later translated into English, the water quality, volume, supply and distribution via the nine active aqueducts, tunnels, cisterns and pipes to Rome, but also relevant laws\(^\text{20,21}\). The detailed descriptions in Frontinus booklet portray e.g. the individual capacity of the aqueducts, the 25 design flows of the bronze nozzles used to deliver fixed amount of water and a list of the 16 previous curator aquarum, from 13 AD, until Frontinus arrival in 97 AD. Frontinus also described the maintenance of the aqueducts e.g. how calcium carbonate deposits should be removed, which otherwise lined the conduit with 1 mm per year.

The technical skills of the engineering and construction are shown in the example of the aqueduct of Nemausus, built around 20 BC. This aqueduct conveyed water approximately 50 km from Uzes to the castellum in the Roman city of Nemausus (Nimes in France), with an elevation difference of 17 m, corresponding to an average slope of 0.34 mm/m along the distance. Settling tanks (piscinae) were located along the aqueducts to remove sediments. In the cities, a fraction of the water was stored in cisterns. The
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cistern Piscina Mirabilis, near Naples, Italy, is one of the largest Roman cisterns, with a capacity of 12 600 m$^3$ of water\textsuperscript{19}. The largest ancient cistern is probably the Yerebatan Saryi or Basilica Cistern in Istanbul, built during 6$^{\text{th}}$ century AD, with a storage capacity of 100 000 m$^3$ water\textsuperscript{22}.

In the distribution systems of water from the aqueducts, lead pipes were used in the junction boxes\textsuperscript{19}. There are however two reasons why lead poisoning of the romans from water pipes did not occur. Firstly, calcium carbonate scaling formed an insulation layer that prevented the water to come in contact with the lead. Secondly, the water was in constant flow through the pipe, giving a short contact time in the last short pipe stretch, outlined in lead.

In the Roman Empire, over 1 600 aqueducts have been described in the Mediterranean basin\textsuperscript{23}, e.g. Figure 1, but over 100 aqueducts were constructed in other parts of the Roman Empire, in today’s Austria, Belgium, Bulgaria, Germany, Hungary, Luxemburg, the Netherlands, Portugal, Romania, Switzerland, Ukraine and United Kingdom\textsuperscript{24}.

Figure 1: Section of the Fontveille aqueduct and a distribution lead pipe built and installed in the 2$^{\text{nd}}$ century in Antibes, France. Photo: B. Björlenius
1.1.3 Post Roman Time in Europe

After the fall of the Roman empire, there was a recession in hygienic conditions and although the aqueducts were still in operation, they were not maintained, resulting in malfunctions. Efforts to restore aqueducts were made in Paris during the 15th century. In the same city, the first sewer was built in 1370, beneath rue Montmartre.

The great conduit in London was an underground channel, constructed in mid 13th century, which distributed spring water more than 4 km into central London. Later, 6 inch lead pipes were installed to transport drinking water in 12 similar conduits systems. A major leap in development was taken in 1580, when Peter Morice applied to city officials for permission to construct a water-wheel and pump under one of the arches of London Bridge. The purpose was to supply the city with cooking water. It was in operation for many years, but it was destroyed in the great fire in 1666, as the great conduits consisted of lead pipes and junction boxes, which melted by the heat. It was however rebuilt and was in operation until early nineteenth century.

In London, public latrines, provided with running water for flushing, were available in the 13th century. The wastewater was directly or finally discharged into river Thames, still serving as the major source of potable water.

On the European continent, the first water conduit to be built after the aqueduct era was in Hamburg in 1370, where drilled hollow logs led fresh spring water, from Altona to several wells in Hamburg. The water conduit was named Katharinen-Brunnen and was in operation for centuries, as can be understood from the reported chemical analysis of the water, dating from 1801.

Some early examples of wastewater treatment come from the 16th and 17th century, with the concept of sewage farms, where crops were irrigated with wastewater, collected in sewer systems. Larger cities like Berlin and Paris implemented large sewage farms. In Paris, irrigation on farmland was established along the river Seine, eventually treating all sewage from the city. In 1948 the sewage farms covered an area of 4487 ha and they produced more than 10% of the vegetables sold at the central market Les Halles. The yields were higher in the sewage farm fields, than in fields at regular farms. Still in the beginning of the 21st century, a fraction of partly treated sewage in Paris was irrigated onto 600 ha of the sewage fields. Later,
the cultivation of only non-food crops on the irrigated fields was considered\textsuperscript{30,33}.

In 1858, the so-called Great stink in London, forced measures to clean the river Thames from the waste from over 2 million Londoners and many industries. It was a great opportunity to implement treatment facilities for the wastewater, e.g. sewage farms, but at this time, only measures for collection and transport of the wastewater in a sewer network was undertaken. The collected wastewater was sophisticatedly discharged and diluted in river Thames, at a few spots downstream the city: In the ends of the two major sewers on the north and south side of river Thames, Abbey Mills and Crossness pumping station were built respectively. The wastewater was stored in reservoirs and pumped at high tide to river Thames. The large-scale project was the solution to the great stink and it saved many humans from new outbreaks of cholera in the 19\textsuperscript{th} century. In 1891 the first treatment step with sedimentation tanks was taken in operation at the Crossness Sewage Treatment Works, which now is one of largest WWTPs in Europe\textsuperscript{34–36}.

\section*{1.2 The development of treatment technologies}

The driving force for the development of municipal wastewater treatment plants (WWTPs) has often been political decisions or successively sharpened legislation, based on technological and scientific conclusions on the influence on human health or the receiving water. The development of the WWTPs has proceeded during more than 150 years, starting from the water works development in 19\textsuperscript{th} century. The wastewater treatment plants were preceded of a period when wastewater was collected and transported in pipes and tunnels away from the cites, to less populated areas, to avoid spreading of diseases and the general pollution in the waters, in the vicinity of the cities.

During the 20\textsuperscript{th} century, WWTPs have been established in many cities. The WWTPs have been extended successively to remove more and more groups of pollutants, starting with coarse material and particles, later bulk organic compound, phosphorus and nitrogen. The individual extensions have often been made during different decades.

In the line of continually improved wastewater treatment, technologies for removal of micropollutants, i.e. inorganic and organic substances with negative effects on the environment, are developed. The negative effects come from the persistent, bioaccumulative and toxic properties of the substances and the name micropollutants comes from their prevailing concentrations in the low micrograms per liter or lower. Currently, focus is mainly on the large
group of pharmaceutical residues, that is mapped and evaluated and, in a few cases, treatment technologies for their removal from wastewater are implemented.

1.2.1 Early development of treatment technologies – removal of organic compounds

Today, most removal technologies for wastewater treatment operate in continuous mode and are designed to have a sufficient average removal efficiency, independently of the hydraulic load. The first developed technologies for biological wastewater treatment were however operated in batch mode.

**Biofilm processes**

An early introduced method for wastewater treatment, applying irrigation over filter materials, like crushed rock or sand, was tested in Paris, Berlin and Manchester and it was first operated in batch mode. In the attached growth of microorganisms, a biofilm containing different bacteria species will developed on the surface of a filter material and the bacteria will break down organic material in the wastewater. When the easily degradable organic material has been degraded, nitrification of the wastewater will take place potentially. The succeeding development of biofilm processes, included trickling filters on initially coarse stone material and later on corrugated plastic sheets\(^{37}\). Starting in the late 1980’s, the development of moving carrier biofilm processes had led to many applications for removal of organic matter and nitrogen\(^{38}\).

**SBR Sequencing Batch Reactor**

The SBR process was developed in the period 1914-1920 when also full-scale SBR-plants were in operation\(^{39}\). The batch process provided great flexibility with fill, reaction, settling, decanting and idle sequences for treating wastewater. Problems with the equipment, high demand for operators attention and the limited possibilities for automation at the time being, limited the use until the end of the 1950’s, when development of SBRs started again\(^{40,41}\). Today SBR technology is used for removal of organic matter, nitrogen and phosphorous in the main stream in some municipal WWTPs, but also in treating side streams, like supernatant from digested sludge\(^{41}\).
**Activated sludge**

The concept of the activated sludge process was presented in 1914, based on research and development that was ongoing from 1882. The essential lab test with activated sludge were performed as batch tests, where a fraction of the accumulated solids was kept in the system between the batches. Oxidation of organic matter and nitrification were observed after five weeks of batch operation. The researchers behind the experiments, Ardern and Lockett named the accumulated solids in the wastewater activated sludge. Further development was intensive in UK and USA and in 1914 the process was operated at two full-scale plants in UK: one plant with fill and draw (SBR-type) and one plant with continues flow. The first plant in USA was taken in operation in 1916. Plants with continuous flow were dominating from the start, 18 of the first 21 plant built during 1914-1927, used continues operation. The activated sludge process is still central in biological treatment all over the world and can contain biological nitrogen and phosphorous removal with different process configurations.

**Activated carbon**

Hindu documents dating from 450 BC refer to the use of sand and charcoal filters for the purification of drinking water. The specific adsorptive properties of charcoal (the forerunner of activated carbon) were first described by Scheele in 1773 in the treatment of gases. Later, in 1786, Lowitz performed experiments on the decolorizing of solutions. In 1862, Lipscombe prepared a carbon material to purify potable water. Activated carbon has gained importance, especially since the mid 1960s, as an adsorptive material in the treatment of municipal and industrial wastewaters. The first full-scale advanced (tertiary) wastewater treatment plant incorporating GAC was put into operation in 1965 in South Lake Tahoe, California.

### 1.2.2 Development and implementation of wastewater treatment plants in Sweden

In Sweden, the first centralized treatment of wastewater was taken in operation in 1904 and consisted of a septic tank for 250 inhabitants, which was constructed in Storängen, Nacka east of Stockholm. The first WWTP with biological treatment with biofilters was taken in operation in Skara in 1911 and the first oxidation ponds were built in 1933-39 in Lund, followed of the first activated sludge process built in Kristianstad 1941. An early and cost effective
design of WWTPs was to construct sedimentation basins for removal of particles and apply anaerobic digestion of the removed sludge, which was installed in Stockholm at Bromma and Henriksdal WWTPs in 1936 and 1941 respectively\textsuperscript{44,45}. The WWTPs in Sweden have successively been extended to meet the effluent standards given to reduce the impact of increasing pollution loads on receiving waters. On average, a new fundamental treatment step has been introduced every 20 years in Sweden\textsuperscript{44}, Table 1. The last two posts have been selected by the author, as they are the most probable process representatives of the last decades.

Table 1. Cycles of implementation of treatment steps in Swedish WWTPs.

<table>
<thead>
<tr>
<th>Decade</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1910’s</td>
<td>Septic tanks</td>
</tr>
<tr>
<td>1930’s</td>
<td>Mechanical treatment</td>
</tr>
<tr>
<td>1950’s</td>
<td>Biological treatment</td>
</tr>
<tr>
<td>1970’s</td>
<td>Removal of phosphorous</td>
</tr>
<tr>
<td>1990’s</td>
<td>Removal of nitrogen</td>
</tr>
<tr>
<td>2010’s</td>
<td>Removal of pharmaceutical residues / Micropollutants</td>
</tr>
</tbody>
</table>

The implementation of the main treatment steps in Swedish has continued from 1930’s until today, Figure 2. Four major causes of the extensions of the WWTPs can be identified\textsuperscript{44}: 1) Primary treatment to remove course material and sludge that polluted lakes and caused odour and esthetic problems 2) Biological treatment to remove organic matter and decrease the number of bacteria – this extension was accelerated by the outbreak of Salmonella in Sweden 1953\textsuperscript{46,47}. 3) Chemical precipitation of phosphorous to reduce the eutrophication in Swedish lakes cause by detergents and increasing population in the late 1960’s 4) Biological nitrogen removal to reduce nitrogen in mainly marine environment which otherwise causes eutrophication and oxygen shortage at sea bottoms along the Swedish coastline in the 1980’s.
An example from activities in the latest cycle is Sweden’s first full-scale treatment step with ozonation for removal of pharmaceutical residues at Knivsta WWTP. This treatment step was designed by the author, based on findings partly presented in this thesis. The ozonation step was designed for 12 000 population equivalents (PE) and was installed and operated at Knivsta WWTP in 2015-2016. The second full-scale plant with removal of pharmaceutical residues in Sweden is currently under operation trials at Nykvarn WWTP in Linköping (Robert Sehlén, personal communication, October 18, 2018). However, the latter plant is the first permanent and largest full-scale ozonation step in Sweden, with a connected population of 145 200 persons, but with a load of organic matter corresponding to 235 000 PE.48.

![Figure 2: Development of Swedish WWTPs. Type of treatment and share of total volume treated.](image)

1.3.4 Number of Swedish WWTPs and their removal efficiencies

The official statistics of water emissions from WWTPs cover WWTPs which have at least 2 000 people connected or a corresponding load of organic material, measured as biochemical oxygen demand (BOD$_7$), of at least 2 000 population equivalents (PE). The sizes of WWTPs are divided into five classes, depending on the number of people connected, Table 2.

The removal efficiencies of phosphorous (P) are independent of the size of the WWTP, due to the applied chemical precipitation, that can be controlled to achieve 90-95% removal of phosphorous, depending on the effluent standard. Removal efficiency for nitrogen (N) is lower for smaller WWTPs, since their biological treatment is designed to fulfil the effluent standards of either 50% or 80% removal of nitrogen. BOD$_7$ have a generally high removal efficiency in
the WWTPs. The removal efficiency increases slightly with increasing size of the WWTP, mainly as a result of measure taken to fulfil higher effluent standards for phosphorous and nitrogen at larger WWTPs\textsuperscript{49}. In addition, there are smaller WWTPs, which are divided into two major groups: those designed for 25-200 PE and those designed for 200 to 2 000 PE respectively. They are however not included in the statistics. These smaller WWTPs are considered to achieve lower removal efficiencies than the larger plants. Furthermore, Sweden has 700 000 on-site sewage facilities (OSSFs) for summer houses and rural areas. The OSSFs have very diverse removal efficiencies depending of type of applied treatment technology and type of substance.

### Table 2: Number of treatment plants in Sweden and removal efficiencies [%] in 2014\textsuperscript{49}.

<table>
<thead>
<tr>
<th>Number of people connected [PE]</th>
<th>Number of WWTP</th>
<th>Total number of people connected [PE]</th>
<th>Removal efficiency of P [%]</th>
<th>Removal efficiency of N [%]</th>
<th>Removal efficiency of BOD\textsubscript{5} [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 000 – 10 000</td>
<td>246</td>
<td>678 682</td>
<td>95</td>
<td>38</td>
<td>93</td>
</tr>
<tr>
<td>10 001 – 20 000</td>
<td>71</td>
<td>602 021</td>
<td>95</td>
<td>56</td>
<td>96</td>
</tr>
<tr>
<td>20 001 – 50 000</td>
<td>64</td>
<td>1 190 827</td>
<td>94</td>
<td>55</td>
<td>96</td>
</tr>
<tr>
<td>50 001 – 100 000</td>
<td>31</td>
<td>1 351 440</td>
<td>95</td>
<td>60</td>
<td>97</td>
</tr>
<tr>
<td>&gt;100 000</td>
<td>19</td>
<td>4 226 783</td>
<td>95</td>
<td>70</td>
<td>97</td>
</tr>
<tr>
<td>Total / Average</td>
<td>431</td>
<td>8 049 753</td>
<td>95</td>
<td>56</td>
<td>96</td>
</tr>
</tbody>
</table>

1.2.3 Centralized water distribution and sewer systems in Stockholm – a case study

In the end of 17\textsuperscript{th} century, Stockholm had 300 wells supplying the Stockholmers with drinking water. The number of wells increased in parallel with increasing population. In general, the wells had good water quality, due to the continuous water outflow along the Brunkeberg esker, which had a high hydraulic capacity to provide ground water. Some wells situated close to tanneries and central shorelines had worse water quality than wells along the esker. In the 17\textsuperscript{th} century a water conduit of hollow logs was constructed leading water to a fountain in the Royal Garden, north of the Royal Castle\textsuperscript{50}. The pandemics of cholera in the 19\textsuperscript{th} century became the driving force for a centralized water distribution system. The 2\textsuperscript{nd} pandemic of cholera reach Stockholm in 1834 and the 3\textsuperscript{rd} in 1850, with yearly cholera outbreaks summertime until 1859. Despite the fact that both the cholera causing toxin,
and the bacteria Vibrio cholerae producing it, was unknown, the spreading of cholera in London was located to well water, by the English physician John Snow. Filippo Pacini wrote a paper on the cholera causing bacteria in 1854, but the discovery seems to have been ignored, or not spread, until Koch published and claimed his identification and discovery of *Vibrio cholerae* in 1880.

In 1861, the distribution of drinking water in a newly built water network began in Stockholm. The distributing of larger and larger volumes of water, led to a plan for a sewer network, presented in 1866, to manage the larger volumes of wastewater. In Stockholm, limitations in the sewer system, did not allow installations of WCs until 1909, where after the installation rate of WCs exploded, in 1915 and 1936, 50 000 and 200 000 WCs had been installed respectively. In 1930, a plan for intercepting sewers, to collect the wastewater from many smaller pipes, were launched and 11 years later, the first wastewater treatment plant for central Stockholm, Henriksdal WWTP was taken in operation. Seven years earlier, Bromma WWTP was taken in operation to serve the western parts of Stockholm.

Today, the Stockholm region has several WWTPs of different sizes, but the trend has been that smaller plants are rebuilt to pumping stations, for feeding larger plants. The reasons were mainly sharpened effluent standards, claiming costly reconstructions in the plants or precautions for securing raw water quality for water works in the region. The specific cost per treated water volume is also lower in larger plants, which promotes the centralization. The treatment plants in the Stockholm region have been extended stepwise during the 20th century, Table 3,

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Mechanical</th>
<th>Biological</th>
<th>Chemical phosphorus removal</th>
<th>Biological Nitrogen Removal</th>
<th>Sand filters</th>
<th>Connected population Peak value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loudden</td>
<td>1950</td>
<td>1969</td>
<td>1970</td>
<td>-</td>
<td>-</td>
<td>50 000</td>
</tr>
<tr>
<td>Eolshäll</td>
<td>1961</td>
<td>1961</td>
<td>1970</td>
<td>-</td>
<td>-</td>
<td>100 000?</td>
</tr>
</tbody>
</table>
The year of implementation of mechanical treatment is also the year of inauguration of the WWTP. Noticeable is the late implementation of biological treatment in Stockholm. Today, wastewaters from Loudden and Eolshäll WWTP’s former catchment areas are pumped to Henriksdal WWTP and Himmerfjärdsverket WWTP respectively.

1.3 Development of legislation on water pollution in Europe

In Sweden, the regulation from 1880 of water rights prohibited in principle the discharge of harmful substances into water. In 1918, the law of water and wastewater was introduced with several amendments; in 1941 concerning judicial control, leading to the obligation of judicial review of point sources. Further strengthening of the legislation by amendments was undertaken in 1955, after the salmonella outbreak in 1953, and in 1970 due to eutrophication. However, the legislation was not sufficient so the Environmental Protection Act of 1969 was launched. Many of these laws were replaced by the Swedish Environmental Code at 1st of January 1999: Miljöbalken 1998:808.

In the European union, the Water Framework Directive (WFD) was adopted in 2000 and it requires that all inland and coastal waters, within certain river basins, must reach at least good ecological and chemical status by 2015 and it defines how this should be achieved through environmental objectives and ecological targets for surface waters. The goal is a healthy water environment with environmental, economic and social considerations taken into account. A list of priority substances, which could threaten human health or ecosystems, was presented in 2000, with the goal to decrease naturally occurring pollutants back to the background values and man-made synthetic pollutants to values close to zero. This first list was replaced by Annex II of the Directive on Environmental Quality Standards (Directive 2008/105/EC), also known as the Priority Substances Directive, which set environmental quality standards (EQS) for the substances in surface waters. In 2012, the European commission proposed to include estradiol, ethinyl-estradiol and diclofenac on the Watch list, which consist of pollutants to be monitored in the environment for eventual inclusion on the list of prioritized substances. In 2015, the antibiotics azithromycin, clarithromycin and erythromycin were added to the watch list.

In Switzerland, an extensive research on micropollutant prevalence, effects in the environment and removal in WWTPs has been undertaken during the last 15 years. The Swiss government decided in 2014 that technical measures must
be implemented over the next 20 years. A selection of 100 WWTPs, out of the existing 700 WWTPs in Switzerland, will be upgraded to remove micropollutants, resulting in more than 80% removal in 50% of the total volume of municipal wastewater in Switzerland\textsuperscript{62}.

Five indicator substances are proposed in Switzerland to represent the micropollutants in wastewater, three APIs: sulfamethoxazole, diclofenac and carbamazepine, one herbicide: mecoprop and one corrosion inhibitor: benzotriazole. All five can be analyzed with the same analytical method\textsuperscript{62}.

The discussion above concerns mainly the concentration levels and the pinpointed, and thereby critical, substances for removal according to the authorities. This has obvious consequences of the selection of the treatment technology, since different methods remove APIs with radically different efficiency\textsuperscript{10,63–65}.

### 1.4 Market of pharmaceuticals

About 4 000 active pharmaceutical ingredients (APIs) are used in human and veterinary medicine worldwide and they are produced by pharmaceutical companies to a combined mass of 100 000 tons per year\textsuperscript{66}. The global market for APIs was valued at USD 134.2 billion in the year 2015 and is estimated to reach a value of USD 239.8 billion by 2025, growing with an average annual growth rate of 6.0%, specifically calculated as Compound Annual Growth Rate (CAGR)\textsuperscript{67}.

In the European Union, about 3000 different APIs are used in human medicine\textsuperscript{68} and in Sweden, approximately 1 200 APIs are authorized in more than 13 700 human and animal pharmaceutical products\textsuperscript{69}. In 2014, human pharmaceuticals in Sweden had a total sales value of 37 829 Million SEK, excluding VAT, corresponding to 1 728 defined daily doses (DDD) per thousand inhabitants and day. For veterinary medicines, the total sales value of 780 SEK millions, excluding VAT, and a total sales volume 3.21 Million packaging. In 2014, the sales value for veterinary medicines corresponded to 2.0% of the total sales value of human and veterinary medicines, where pets represents over 50% of the veterinary consumption\textsuperscript{70}.

### 1.5 Routes/pathways of pharmaceuticals in the environment

APIs in the aquatic environment originate from several sources, mainly from human use of medicines, Figure 3. The APIs or conjugated forms of the API are subsequently excreted into urine and feces, which are transported to WWTPs (mWWTPs in Figure 3) or OSSFs, where some APIs are removed, but
most substances remain, to different extents, in the treated wastewater, which is discharged to surface or ground water.

In Sweden, 97% of the human medicines (reported as number of DDDs) are consumed in non-institutional care, only 3% of the human pharmaceuticals are used in hospital care\textsuperscript{71}. The hospitals are nevertheless discussing on-site treatment of wastewater from some clinics to remove e.g. antibiotic residues from wastewater with high concentrations, but in Sweden today, the hospital wastewaters are treated in municipal WWTPs.

At API production sites outside Europe, discharges of untreated or partly untreated industrial wastewater have been reported\textsuperscript{74}. In a specific case, the concentrations of ciprofloxacin in wastewater exceeded the maximum therapeutic dose in human plasma. The concentrations of e.g. losartan,
cetirizine, metoprolol and citalopram were typically 1000 higher in the industrial wastewater, than in municipal wastewater in general. In Europe, industrial WWTPs (iWWTPs) generally treat the wastewater from the API production sites.

The relative importance of veterinary medicines, as sources of APIs in the aquatic environment, are lower in Sweden, than in some other countries. Within the EU, it is forbidden to use antibiotics for growth-promoting purposes in all animal farming, which also includes fish farming. However, in Czech Republic, Denmark, Finland and the Netherlands the usage of antibiotic substances increased as sales per kg of animals in 2009, compared to 2005. They are often categorized as “therapeutic” antibiotics, e.g. in Denmark the antibiotic dosage per pig increased by 24% between 2001 and 2008 and antibiotics are used systematically to control diseases in intensive farms and involved in mass medication. Use of antibiotics in production of poultry, pigs and cattle are still widespread in the US and elsewhere outside the EU. An estimation shows that the global consumption of antimicrobials increased by 67% between 2010 and 2030.

The APIs of veterinary origin are excreted in pet excrements, manure or fish debris. The pet excrements and fish debris will, to a large extent, rapidly come in contact with the aquatic environment. The manure from farms will be used as fertilizer on soil, where some APIs can be adsorbed and removed, mainly by microbial activity. Potentially, some low adsorptive APIs will migrate to surface and groundwater.

The collection of unused medicines is an important upstream measure to avoid discharge of APIs into the aquatic environment. In Sweden, the estimated volume of unused medicines is 5%. All pharmacies in Sweden take the unused medicines in return, free of charge. The collected medicines are incinerated at higher temperatures, than conventional household garbage, which is incinerated at 700-850°C. The waste incineration EU directive 2000/76/EC states that the temperature by incineration of hazardous waste containing 1% Cl must be 1100 °C during 2 seconds. Pharmaceutical waste must be incinerated at >1000 °C or for halogenated content >0.5% at 1200°C. The waste of used medicines in household garbage is thus not recommendable, but it is still better than flushing them into the sewer.

The sludge from the WWTPs is incinerated at 750-1000°C or is used as fertilizer in agriculture. Theoretically the incineration temperatures are too low to destruct all APIs. Like in the case of manure, APIs in sludge from WWTPs can be adsorbed and removed, but some APIs will migrate to surface
and groundwater. APIs in treated wastewater, ending up in surface water, might be degraded by natural UV light, but some APIs will find their way to drinking water, since traditional treatment of surface water in waterworks, do not include ozonation or activated carbon treatment, which otherwise would remove them to a large extent. However, in more densely populated areas with water scarcity or raw water with low quality, waterworks have been extended with treatment of micropollutants, including APIs.

### 1.6 Reported effects of pharmaceuticals in the environment

Pharmaceutical residues have been reported to cause optically observable adverse effects in wild organisms. Feminization of male fish, due to natural and synthesized estrogens, was the first observations from downstream locations of municipal WWTPs in England and was reported in the 1970s, in a few scientific paper or reports, which increased the interest and awareness of hormones in the environment among researchers and the public. Additional scientific studies were undertaken in the 1970s, e.g. of the fate of some veterinary APIs (phenothiazine, sulfamethazine, clopidol, and diethylstilbestrol) in aquatic model ecosystems and on APIs and their metabolites as environmental contaminants. During the 1980s, mapping of APIs in the effluent of WWTPs continued and since the late 1990s interests into the fate of pharmaceuticals in the WWTPs and in the environment has accelerated. The most important reason for the interest are the reports of observed effects of pharmaceuticals on water-living organisms; hermaphrodite fish in ponds, downstream of a WWTP, initiated a study with caged fish in the effluent from 15 WWTP in England, showing dramatically increased levels of vitellogenin, a precursor protein of egg yolk, also in male fish. Vitellogenin has since then been an important biomarker for estrogenic contamination of the aquatic environment. Concentrations of natural steroidal estrogens in British rivers were sufficient to increase vitellogenin synthesis observed in male fish.

In 2004, two studies showed on effects of diclofenac on organisms at environmental relevant concentrations. In the first study, rainbow trout (Oncorhynchus mykiss) was observed to bioconcentrate diclofenac in liver, kidney, gills and muscle. Based on the observed effects on kidney and gills at a water concentration of 5 µg/L, the no observed effect concentration (NOEC) of diclofenac was proposed to be 1 µg/L. Further evaluation showed on effects also in tests with 1 µg/L, proposing the NOEC to be lower than 1 µg/L.
i.e. corresponding to typical concentration in effluent at Swedish WWTPs. In a recent paper, the low NOEC values from 2004 were disputed as the moderately reduced observed growth-rates were interpreted as artefacts in the new study, suggesting a much higher NOEC value of 320 µg/L.

Another route for diclofenac in the environment was discovered on the Indian subcontinent where the population of three spices of vultures (Gyps bengalensis; G. indicus and G. tenuirostris) declined by 34-95% during the period 1990s-2003 and the death of the vultures was associated with renal failure resulting in visceral gout. Residues of veterinary diclofenac in animal carcasses were proposed to be responsible for the decline in vulture populations. In Pakistan, veterinary diclofenac is sold without prescription for treatment of cattle, which after death, are left for scavengers like vultures to remove.

Further examples demonstrating the effects of APIs on aquatic organisms, at environmentally relevant concentrations, show that the lowest effect concentration of fluoxetine and ibuprofen on the activity of Gammarus pulex, an amphipod crustacean, was 100 ng/L and 10 ng/L respectively. The lipid regulator Gemfibrozil was bioconcentrated in goldfish (Carassius auratus) and the plasma concentration of testosterone was reduced by over 50% in the fish.

Antibacterial drugs, as environmental contaminants, were discussed in the early 1970s. Antibiotic substances are essential to treat bacterial infections, but they are also used in the less requisite application on livestock in agriculture. The extended use and misuse of antibiotics has increased the prevalence of antimicrobial resistance (AMR) e.g. among clinically relevant bacteria. The spreading of AMR is a substantial threat to human health and many initiatives and measures are in progress to decrease the spreading of AMR and to extend the usability and life-time of the available antibiotics. No novel classes of antibiotics have been presented to the treatment of diseases since 1995. One reason for the lack of new classes is the decrease in budgets for antibiotic R&D in the major pharmaceutical companies. However, creative design of new molecules is possible within the existing antibiotic classes to improve their therapeutic properties.

Antibiotic residues in wastewater from production sites are considered to be one source for induction of AMR. In municipal WWTPs, the continuous input of AMR bacteria from connected humans has been considered to be much more important than the inflow of antibiotic residues. The concentrations of antibiotics in municipal wastewater are very low compared
to therapeutic concentration, but also low compared to the concentration in wastewater from hospitals\textsuperscript{97}. In a recent study, the concentrations of ciprofloxacin and tetracycline in the influent to three Swedish WWTPs, exceeded the predictive threshold concentrations for resistance selection. However, no enrichment of any particular class of antibiotic resistant genes in the WWTPs were seen\textsuperscript{98}.

Synthesized progestins, a class of contraceptive pharmaceuticals have recently moved into focus in the field of ecotoxicology. Natural progestins are involved as reproductive hormones in all vertebrates. In total 20 synthesized progestins are in use in human and veterinary medicine\textsuperscript{99,100}. The synthetic progestins, used for contraception so far, are structurally related either to testosterone or to progesterone. Several new progestins have been designed to minimize side-effects. The most potent progestins can be used at very low doses\textsuperscript{99,101}. This indicates that low environmental concentrations could have an effect on water living organisms. Two progestins, levonorgestrel (LNG) and norethindrone (NET) have been identified as highly potent androgenic pollutants in the aquatic environment, at low ng/L level\textsuperscript{100,102}.

A recent study showed that oxazepam altered the behavior and feeding rate of wild European perch (\textit{Perca fluviatilis}), at environmental relevant concentrations, in effluent-influenced surface waters. The change in behavior, in form of increased activity and reduced sociality, will probably have ecological and evolutionary consequences, as well as that the increased feeding rate can influence the structure of the aquatic community\textsuperscript{103}.

The substances now discussed are hitherto the most important examples of pharmaceuticals giving adverse effects at environmentally relevant concentrations. Together with several hundred other APIs, they can be found in the Wikipharma database containing publicly available ecotoxicity data for pharmaceutical substances\textsuperscript{104,105}.

\textbf{1.7 Environmental risk assessment (ERA)}

ERA is an important tool in the work chain, from research on environmental effects, to action taken to prevent the environment from harmful substances; Research $\rightarrow$ Risk assessment $\rightarrow$ Risk management\textsuperscript{104}.

In the ERA, identification and characterization of environmental risks form the basis for a decision of the risk connected to a specific chemical, to prevent
unacceptable harm to the environment, taking into account economic, engineering, political and social information\textsuperscript{106}.

Since 2006, environmental risk assessments are required for all new marketing authorization applications for APIs. It includes recommendations of using OECD test protocols for physical-chemical, fate and effects studies in the first phase. If potential risks have been identified in the first phase, then additional OECD tests should be performed. However, a market introduction is not prohibited, despite detected negative impacts in the tests\textsuperscript{107}.

In 2016, ten years after the release of the guidance for environmental risk assessment of human pharmaceutical products, ten recommendations to improvements of the assessment were published by the MistraPharma research project team\textsuperscript{108}.

1. Include substances introduced to the market before 2006
2. Requirements to assess the risk for development of antibiotic resistance
3. Jointly performed assessments by several companies
4. Refinement of the test proposal
5. Mixture toxicity assessments on active pharmaceutical ingredients with similar modes of action
6. Use of all available ecotoxicity studies
7. Mandatory reviews at regular intervals
8. Increased transparency
9. Inclusion of emission data from production
10. Inclusion of environmental risks in the risk-benefit analysis

The published guidelines have been presented for several stakeholders, including the European Parliament and national water and wastewater organizations.

1.7.1 Ecotoxicology tests of wastewater

Treated wastewater contains complex mixtures of micropollutants, raising concerns about effects on aquatic organisms. The addition of advanced treatment steps could for some processes contain, or potentially produce, effluents affecting exposed organisms by known or unknown modes of action\textsuperscript{109}.

Ecotoxicological assays for studies on the effect of micropollutants have for many years focused on organism’s individual level, with endpoints like
individual growth, reproduction and mortality. Today, the biomarker responses on biochemical and cellular systems are frequently used, being more sensitive and with faster response to changes in the test environment. Many ecotoxicological biomarkers originate from biomedical sciences and many of the mechanisms are conserved in mammals and different organisms, allowing the biomarkers to indicate the same or similar processes on biochemical or cellular level\textsuperscript{110,111}. However, the conserved number and similarities to mechanisms in human differ in different species e.g. zebrafish \textit{Danio rerio} has orthologs to 88\% of the human drug targets, while 63\% are conserved in \textit{Daphnia pulex}, 36\% in green algae \textit{Chlamydomonas reinhardtii} and 19\% in \textit{E. coli}\textsuperscript{112}.

The combination of ecotoxicological assays and studies of biomarker responses are still valuable for development of biomarker assays and the modelling of mechanisms at different levels in the organisms, but also in the evaluation of the effects of individual and complex mixtures of chemicals like the situation in municipal wastewater\textsuperscript{110}.

1.7.2 Biomarkers

The term biomarker is generally almost any measurement reflecting an interaction between a biological system and a potential chemical, physical or biological hazard. The measured response can be functional, physiological, biochemical at the cellular level or a molecular interaction\textsuperscript{113}.

One of the most commonly used biomarkers in studies of the aquatic environment is the induction of vitellogenin in male and juvenile fish, as a result of exposure to estrogenic compounds. As a complement, the induction of the glue protein spiggin is the only known quantitative, molecular biomarker for androgenic compounds in fish. Only the male fish produces the spiggin for nest building and a production of spiggin in female fish is induced by exposure to exogenous androgenic substances\textsuperscript{114}.

The Cytochrome P\textsubscript{450} (CYP) monooxygenases are members of the hemoprotein superfamily and are involved in metabolism of endogenous compounds such as steroids, fatty acids, and prostaglandins and exogenous compounds such as chemical pollutants including pharmaceuticals. Fish has been reported to have 18 families of CYP genes (CYP1, CYP2, CYP3, CYP4, CYP5, CYP7, CYP8, CYP11, CYP17, CYP19, CYP20, CYP21, CYP24, CYP26, CYP27, CYP39, CYP46 and CYP51) and numerous subfamilies. CYPs catalyze the conversion of lipophilic substances to more water-soluble substances.
primarily by oxidation\textsuperscript{115}. The mRNA expression of CYP1A is known to be highly inducible by a number of aryl hydrocarbon receptor (AhR) agonists like PAHs and other planar aromatic (aryl) hydrocarbons in various fish species\textsuperscript{116}. One non-target ecotoxicity test, the \textsuperscript{1}H NMR (proton nuclear magnetic resonance spectroscopy) metabolomics of fish blood plasma can be used to explore responses not identified by more targeted (chemical or biological) assays\textsuperscript{109}.

### 1.8 Evaluation of chemical data - Deconjugation and accuracy

Pharmaceutical residues appear in concentrations of ng/L to µg/L in wastewater and they can be in form of parent (original) substances or conjugated substances. For some APIs, higher concentrations are reported in the WWTP effluent, than in the influent, in corresponding samples, at the same WWTP. This negative removal can be a result of either deconjugation, analytical uncertainty or improper sampling. Deconjugation means cleavage of a conjugate of an API, with a molecule like glycine, glucuronic acid or glutathione. The conjugates are produced in humans to facilitate excretion of APIs with urine or bile\textsuperscript{63,117}. In the WWTPs, bacterial enzymes are active in the deconjugation, which increases the concentrations of APIs in the effluent, compared with the influent, for some slowly degradable substances.

The analysis of pharmaceutical residues demands advanced methods, but no standardized analytical method is available, although several methods have been reported. Most of the APIs are present at ng/L level and considerable analytical uncertainties follow with the low concentrations. To evaluate the accuracy of the different in-house analytical methods, which all use SPE sorbents, chromatographs and mass spectrometers, an intercalibration was performed at five Swedish laboratories in 2008\textsuperscript{118}. The intercalibration showed that the distribution in results between the laboratories, for the same API and water, is higher than the spread between replicates within the same laboratory, which means that the laboratories perform reproducible results, but the results are not the same, i.e. different systematic errors are built into the individual laboratory method. The variation of the results was significantly greater for APIs that are poorly removed, or not removed at all, in the WWTPs. Interestingly, for APIs with poor removal (<30%), an analytical error of 20% can lead to a calculation result of negative removal and be mistakenly interpreted as deconjugation of API metabolites. The cause of systematic errors lies primarily in complexity in composition of the
wastewater. Interfering particles and other substances cause a matrix effect, particularly in samples of influent wastewater\textsuperscript{118}.

Ion suppression is one form of matrix effect that negatively affects detection capability, precision, and accuracy. Ion suppression is caused by irrelevant substances from wastewater, often reducing the signal for the APIs, resulting in improperly lower values. However, depending upon the type of sample, it also can be observed as an increase in the response of the desired analyte. Strategies have been developed to validate the presence and quantitatively calculate the extent of ion suppression\textsuperscript{119}.

Another factor that can contribute to different results from different laboratories is the distribution of APIs between water and particles in the samples. Some APIs tend to bind to particles and the pretreatment of the samples, by filtration (0.45-1.6 $\mu$m) of influent wastewater, which almost all laboratories performed prior to concentration and analysis, may have resulted in the determination of the waterborne API component only. The filtration also means that reported levels in WWTPs’ influent samples often are lower than the actual levels. This in turn leads to the fact that too low removal efficiencies over the WWTPs are calculated for certain APIs \textsuperscript{118}.

Improper sampling of a non-constant flow of wastewater results in bad raw data. Normally a diurnal variation in substance concentration and wastewater hydraulic flow occurs, but also mass flow of pollutants has a diurnal variation in the WWTPs. This is also the case for APIs\textsuperscript{120--122}. Grab samples from influent and effluent wastewater, taken without regard to the hydraulic retention time in the WWTP, will probably cause errors in calculations of removal efficiencies due to non-correlating samples. Flow proportional 24h composite samples normally taken at many WWTPs are more likely to be a good base for calculations, although they are not corresponding regarding the same water portion, but they cover the daily, often repeated, diurnal variation. Sampling points in the WWTP must be selected so internal streams like supernatant from the dewatering of digested sludge will not be discharged to the influent wastewater, prior to sampling\textsuperscript{123}.

1.9 Pharmaceuticals in WWTPs and the aquatic environment

The municipal wastewater treatment plants are designed to treat household wastewater regarding suspended material (particles), easily biodegradable organic matter, phosphorus and nitrogen and the WWTPs are not designed to specifically remove micropollutants like pharmaceutical residues. However, some APIs are partly or fully removed by sorption and biological degradation
or transformation in the biological treatment in the existing WWTPs\textsuperscript{64}. For this introduction, the median concentration of APIs in the influent, effluent and the resulting removal efficiency in Swedish WWTPs were determined, based on published data of pharmaceutical residues from different Swedish authorities\textsuperscript{63,124–127}. The Swedish Environment Protection Agency, County Councils and municipalities have sampled the influent and effluent of many WWTPs, to get an idea of the situation of pharmaceutical residues in wastewater. However, only a few compilations of the raw data on pharmaceutical residues have been done previously and then on subsets of the data. In the following paragraphs, the results of the compilation made for this thesis is presented and discussed.

1.9.1 Occurrence of APIs in the influent in Swedish WWTPs

Samples from the inlet to WWTPs have been taken by several authorities, e.g. Swedish Environment Protection Agency and County Councils, to study the concentrations and load of pharmaceutical residues on the WWTPs, but also to enable calculation of removal efficiencies in the regular WWTPs. The available data used in the compilation come from in total 91 Swedish WWTPs including 85 samples from influent wastewaters and 150 samples from WWTPs effluents\textsuperscript{63,124–127}. One explanation to the lower number of inlet samples can be that the authorities want to get an idea of the remaining APIs in the effluent to estimate the pollution load from the WWTPs on the environment. The reported samples were taken as grab samples or composite samples and they were analyzed by one of a few external labs that can offer analysis of pharmaceutical residues in Sweden.

The concentrations of API in influent wastewater differed a lot and they are therefore presented in a logarithmic diagram to make a visual comparison feasible, Figure 4. In the influent to the WWTPs, seven APIs had a median concentration over 1 µg/L, whereof the painkiller paracetamol reached the highest concentration 69 µg/l, followed by two other painkillers ibuprofen and naproxen.
Next in concentration order was furosemide, a diuretic API, followed by another painkiller, ketoprofen. The last two substances, with concentrations exceeding 1 µg/L, were the beta-blockers atenolol and metoprolol. The four painkillers in “top 7” are all non-steroidal anti-inflammatory drugs (NSAIDS) with the exception of paracetamol. The remaining concentration level groups of APIs represent several therapeutic classes in each group. Data showed that 22 APIs had a median concentration between 100 and 1000 ng/L. In the range 10-100 ng/L, 19 APIs were recorded, and 11 APIs had concentrations in the range of 1 to 10 ng/L. Ethinyl estradiol was the only API quantified below 1 ng/L, through the specific analytical methodology. The concentration levels reflected the consumption and excretion of parent substance after consumption: The DDD is for paracetamol 3000 mg and for ethinyl estradiol 0.035 mg. The DDDs for the two APIs differs by a factor of 100 000 and the concentration in the influent differs by a factor of 200 000, thus being in the same range. The excretion from humans of paracetamol and ethinyl estradiol is reported as 2-3% and 2-40% respectively. In conclusion, many of the APIs analyzed for, were found in the influent to the WWTPs, with varying concentrations, reflecting the API’s DDD and the total use.
1.9.2 Occurrence of APIs in the effluent from Swedish WWTPs

The national data described above were compiled to evaluate the concentrations of APIs in the effluent from Swedish WWTPs\textsuperscript{63,124–127}. In parallel with the case of API concentrations in the influent, the concentrations of APIs in the effluent are presented in a logarithmic diagram to facilitate comparisons, Figure 5. The effluent concentrations are related to the inlet concentrations and the different processes in the WWTPs, including adsorption and biological transformation as oxidation and deconjugation. The removal of different APIs varied a lot in the existing WWTPs and combined with the large range of influent concentrations, the effluent concentrations showed a large range as well, however not as broad as the inlet concentrations. The order of substances sorted in concentration magnitude has changed in comparison with the order of substances in the influent, which indicates the substances are removed to different degrees.

![Figure 5. Median concentration in effluent from Swedish WWTPs. Notation #W corresponds to the number of WWTPs sampled and #S corresponds to the number of samples with quantified concentrations.](image)

From the top 7-list of high concentrations in the influent, furosemide, metoprolol and atenolol remained at approximately the same concentration in the effluent, showing the low removal efficiencies in existing WWTP. Data provided concentrations of four of the six APIs and hormones on the WFD
present watch list; diclofenac, erythromycin, 17-beta-estradiol (E2), and 17-alpha-ethinylestradiol (EE2)\textsuperscript{58}. The median concentration in the effluents for these substances were 251, 220, 0.5 and 0.3 ng/L respectively. Proposed annual average environmental concentrations in freshwater (marine water) for diclofenac, erythromycin are set to 100 (10) ng/L and 200 (20) ng/L and for 17-beta-estradiol and 17-alpha-ethinylestradiol 0.4 (0.08) ng/L and 0.035 (0.0070) ng/L, respectively, leading to the conclusion that as long that the effluents of the Swedish WWTPs is diluted by a factor of 10 in the receiving water, the environment concentrations will be lower than the proposed environmental quality standards EQS\textsuperscript{131–134}.

In conclusion, many of the APIs are still present in the effluent from the WWTPs, which shows that the removal capacity is limited in regular WWTPs and that pharmaceutical residues are discharged into the aquatic environment via WWTPs. The authorities use the data to map the magnitude of the discharged pharmaceutical residues and, in a few cases, to model the environment concentrations of pharmaceutical residues in watersheds.

1.9.3 Removal efficiencies of APIs in Swedish WWTPs and the presence of APIs in the aquatic environment and in drinking water

The removal efficiency describes to what extent the APIs have been removed in a regular municipal WWTP. The statistical processing of national raw data of influent and effluent concentrations presented above\textsuperscript{63,124–127}, showed the removal efficiencies for a selection of APIs in Swedish wastewater treatment plants. Raw data were of different origin, from different years and analyzed at different laboratories. WWTPs represent both small and large plants with different process design and the removal efficiencies are calculated in the corresponding samples. In the case of a lower concentrations in the effluent than the limit of quantification, LOQ, the removal efficiency was calculated on the concentration LOQ/2 in the effluent. A big difference in removal efficiencies was observed whether the sewage treatment plant has biological nitrogen removal or not Figure 6.
Nine APIs had a negative removal in the studied WWTPs due to deconjugation, improper sampling and analytical limitations, which can explain the results as discussed earlier. Sixteen APIs were removed more than 80%, including three of the four painkillers with high concentrations in the influent. Paracetamol and ibuprofen were removed more than 99%. In conclusion, the removal efficiencies in the regular WWTPs differed much between the APIs. The average removal for all APIs was 44% and the median removal efficiency was 52%. The compilation showed that the removal efficiency of APIs was limited in regular municipal WWTPs and measures must be taken if improved removal efficiencies of APIs are required.

This evaluation showed that APIs were present in regular municipal wastewater, both in the influent and effluent waters. Thereby, there is a risk for accumulation of APIs in the receiving water bodies and ultimately a potential effect on the living organisms in the receiving water.
Pharmaceutical residues in the aquatic environment

More than 600 active pharmaceutical ingredients, their metabolites or transformation products have been found in aquatic systems globally, due to a combination of worldwide usage and low removal efficiency in wastewater treatment plants (WWTPs), or a complete lack of WWTPs. In surface waters, concentrations of pharmaceuticals usually range from low ng/L to low µg/L. The concentration range shows that APIs are true micropollutants. Discharges from pharmaceutical production sites, have been shown to result in concentrations as high as mg/L in receiving surface waters.

Pharmaceutical residues in drinking water

The concentrations of pharmaceutical residues in drinking water are more than 1000-fold below the minimum therapeutic dose, which is the lowest clinically active dosage. The measured trace concentrations of pharmaceuticals in drinking water are deemed unlikely to pose risks to human health, due to the safety margin between the concentrations quantified and the concentrations of lowest clinically active dose. Based on the current low concentrations of carbamazepine and other pharmaceutical residue in drinking water, WHO has concluded, that impact on human health is very unlikely from drinking water and consequently, no WHO guideline values for pharmaceuticals in drinking water will be formulated for the time being.

1.10 Potential technologies for removal of pharmaceuticals

To increase the removal efficiencies of pharmaceutical residues in regular municipal WWTPs, additional treatment measures must be applied. Biological treatment, separation and chemical oxidation have been suggested as main process categories for removal of pharmaceuticals from wastewater. The specific treatment technologies or unit operations were divided into these categories in Table 4 and are further described in detail below, to show the principles, process conditions and removal efficiencies of pharmaceutical residues.
Table 4: Regular and advanced treatment technologies

<table>
<thead>
<tr>
<th>Category</th>
<th>Unit operation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological treatment</td>
<td>Conventional Activated Sludge (CAS)</td>
</tr>
<tr>
<td></td>
<td>Aerated granular sludge (AGS)</td>
</tr>
<tr>
<td></td>
<td>Membrane Bio Reactor (MBR)</td>
</tr>
<tr>
<td></td>
<td>Carrier Biofilm Systems (MBBR)</td>
</tr>
<tr>
<td></td>
<td>Enzyme transformation (ENZ)</td>
</tr>
<tr>
<td></td>
<td>Transformations by microorganisms (Algae or Fungi)</td>
</tr>
<tr>
<td>Separation processes</td>
<td><strong>Sorption:</strong></td>
</tr>
<tr>
<td></td>
<td>Activated Carbon (AC)</td>
</tr>
<tr>
<td></td>
<td>Adsorption on Sludge</td>
</tr>
<tr>
<td></td>
<td><strong>Membrane filtration:</strong></td>
</tr>
<tr>
<td></td>
<td>Nano Filtration (NF)</td>
</tr>
<tr>
<td></td>
<td>Reverse Osmosis (RO)</td>
</tr>
<tr>
<td>Chemical oxidation</td>
<td>Ozonation (O₃)</td>
</tr>
<tr>
<td></td>
<td>Ultra Violet Light + Hydrogen Peroxide (UV/H₂O₂)</td>
</tr>
<tr>
<td></td>
<td>or TiO₂</td>
</tr>
<tr>
<td></td>
<td>Ozonation + Hydrogen Peroxide (O₃/H₂O₂)</td>
</tr>
<tr>
<td></td>
<td>Chlorine dioxide (ClO₂)</td>
</tr>
<tr>
<td></td>
<td>Peracetic acid (PAA)</td>
</tr>
</tbody>
</table>

Potential of biological treatment for removal of pharmaceuticals

Enhanced biological degradation of pharmaceutical residues can be achieved by increasing the sludge age in existing biological treatment or by treating the effluent by biofilm processes (biofilm systems, trickling filters, slow sand biofilter). Generally, the average removal of pharmaceutical residues by biological treatment is not sufficient, but for some APIs, biological treatment gives high removal efficiencies\textsuperscript{10,109,140,141}. Processes involving enzymes, algae and fungi are out of scope of this thesis.
1.10.1 Conventional Activated Sludge (CAS)

The activated sludge process is a system where suspended growth of bacteria form settling flocs with incorporated organic and inorganic material. The flocs are kept in suspension by aeration, which also supplies the bacteria with oxygen for degradation of organic material. The aeration also strips the produced carbon dioxide and other gases from the wastewater. The continuous flow of wastewater transports the flocs, from the activated sludge basin, to a subsequent sedimentation basin, where the flocs settle to the basin bottom, from which approximately 98% is recirculated back to the inlet of the activated sludge basin. The remaining 2% of the flocs are removed from the activated sludge step in form of an excess sludge for further processing. The removal efficiency of organic matter is generally very high, between 90 and 95 percent measured as BOD$_7$ (biochemical oxygen demand for 7 days). Adsorption of some APIs to the activated sludge contributes to the removal of pharmaceutical residues over the biological treatment step. Furthermore, activated sludge degrades some pharmaceutical residues and sludge age is an essential parameter for the removal efficiency\textsuperscript{10}. Aerobic sludge ages exceeding seven days resulted in higher efficiencies for some APIs, than aerobic sludge ages of less than three days, but still the average removal efficiency of pharmaceutical residues was limited to approximately 50%\textsuperscript{10,142}.

1.10.2 Aerated granular sludge (AGS)

The performance of the conventional activated sludge process is dependent of sludge with good settling properties. Aerated granular sludge (AGS) consists of biologically active granules with high settling velocities, 30-40 m/h\textsuperscript{143}, in comparison with typical settling velocities of activated sludge, 0.5-8 m/h, the latter range covers different sludge concentrations and sludge morphologies\textsuperscript{144,145}. The aerobic granules are most commonly formed in a sequencing batch reactor (SBR) with process conditions that retain the aerobic granules, but the setup let activated sludge flocs to be washed out of the system\textsuperscript{146}. The AGS technology enables the design of compact WWTPs with simultaneous removal on organic material, nitrogen and phosphorous\textsuperscript{147}. In the few published papers on removal of pharmaceuticals by AGS, the reported removal efficiencies are for some APIs inconsistent with the removal efficiencies in conventional activated sludge\textsuperscript{148–150}. The removal efficiencies were however not evaluated in parallel at the same site and the number of studies is limited due to the recently developed AGS technology.
The sorption of APIs to the AGS was substantial in one study, 50-85% of the APIs in the feed ended as adsorbed substances\textsuperscript{148}.

1.10.3 Membrane Bio Reactor (MBR)

The separation of the active sludge in an activated sludge processes usually takes place in a sedimentation basin and is driven by gravity. During the past 20 years, membrane technology in form of micro- or ultrafiltration has been used more and more for the separation of activated sludge, due to better separation of suspended solids, also at higher mixed liquor suspended solids (MLSS) concentration, than in conventional activated sludge. The high MLSS concentration makes the treatment step, entitled membrane bioreactor (MBR), compact with a smaller footprint. Increasing the MLSS in combination of an increased sludge age has been shown to improve the removal of pharmaceutical residues by 10-20\%\textsuperscript{10,109}.

The activated sludge is separated over a membrane by cross flow filtration, which means that a small flow of particulate-free water is taken out through the membrane surface, while a much higher flow passes the membrane tangentially. The large flow is created by air, blown from beneath the membrane modules. The large amount of air requires input of more electrical energy, but low energy systems are developed. Furthermore, chemical cleaning is needed regularly to avoid lower hydraulic capacity due to fouling and scaling. The cleaning frequencies vary for different membrane brands and strategies are still developed to decrease the fouling. There are two main types of membranes: flat sheet and hollow-fibre membranes. The membrane bioreactors are implemented in full-scale worldwide\textsuperscript{10}.

1.10.4 Biofilm Systems (MBBR)

In biofilm processes, different bacteria species are attached on surfaces of a support material like sand, crushed rock or plastic. In the biofilm, the bacteria will decay organic material in the wastewater. When the easily degradable organic material has been degraded, potentially nitrification of the wastewater will take place. The attached growth makes the bacteria more protected from variation in flow and wastewater quality than the bacteria in many suspended growth applications. An important process parameter to monitor in biofilm applications is the mass transport of organic substances, nutrients and gases, to and from the biofilm. Limitation in diffusion due to
e.g. the biofilm thickness will slow down the reaction rates. Layers with different redox potential are evolved in a biofilm, which facilitates e.g. both nitrification and denitrification to take place. Today, the availability of moving plastic carriers for biofilm has resulted in many applications for removal of organic matter and nitrogen, as alternatives to activated sludge, which for long was the dominating process for biological treatment\cite{37,38,151}. One hypothesis for biofilm systems is that the long sludge age for the attached bacteria will facilitate the establishment of a diverse microbial community that can degrade also slowly degradable substances, like some pharmaceutical residues\cite{10}. Recent research has shown that biofilm systems degrade some pharmaceutical residues more efficiently than activated sludge\cite{152,153}.

1.10.5 On-site sewage facilities - Prevalence and removal of pharmaceuticals

In a Swedish study, two on-site sewage facilities (OSSFs) with soil filter bed systems, showed higher removal efficiencies of 15 pharmaceutical residues, than four large Swedish municipal WWTPs. On the contrary, diclofenac and ketoprofen had significantly lower removal efficiencies in the OSSFs, than in the WWTPs\cite{154}. Another Swedish study, with pooled samples from eight soil filter beds, showed that the removal of diclofenac was on average 87% in the soil beds, compared to the achieved 34% removal efficiency in the reference WWTP\cite{155}.

The removal of organic substances in soil filter bed involves a biofilm process where bacteria are attached to soil particles. A comparison of removal rates of diclofenac and ketoprofen in activated sludge and biofilm processes, performed in parallel in lab scale batch tests, showed that the biofilm system removed diclofenac and ketoprofen faster than the activated sludge\cite{156}.

The residence time for the bacteria in a soil filter bed is longer than in a regular WWTP with activated sludge\cite{154}. In the soil filter bed, the bacteria are not removed, except with occasional losses of biofilm to the effluent water, in contrary to WWTP, where the sludge age is generally controlled in an interval of 3-25 days, depending on applied process and conditions such as wastewater temperature. The longer hydraulic and bacteria retention time seems to give the higher removal efficiency in the soil filter beds\cite{154}. One conclusion from the study is that OSSFs both can remove some pharmaceutical residues better than a WWTP, but also give a lower removal
of other pharmaceutical residues. In the latter case, this leads to a discharge of diffuse pollution of pharmaceuticals over large land areas\textsuperscript{154}.

Prevalence and removal of micropollutants in OSSFs were extensively studied in a recently ended Swedish research project, “Redmic”, where screening and evaluation of add on methods to remove pharmaceuticals, personal care products, pesticides, phosphorus-containing flame retardants and PFAS were performed. In total 79 micropollutants were successfully identified whereof a prioritized set of 20-26 substances was further evaluated in fate studies, monitoring in watersheds and used as model substances in the development of treatment technologies.

Concentrations of micropollutants were similar in influents and effluents of OSSFs and WWTPs, respectively. Overall, the removal rates of micropollutants in OSSFs and WWTPs were rather low. Removal of PFASs and PFRs (phosphorus-containing flame retardants) were higher in the OSSF’s package treatment and the WWTPs, while the selected pharmaceuticals and personal care products (PPCPs) were more efficiently removed in the soil beds in the OSSFs\textsuperscript{155,157,158}.

1.10.6 Factors/processes contributing to removal of APIs in regular WWTP

There are three main conceivable mechanisms to reduce pharmaceutical residues in today's sewage treatment plant: Stripping to air, adsorption to particles (sludge) and biochemical transformation (biodegradation)\textsuperscript{64}.

\textit{Stripping}

The loss of APIs to the air in the WWTPs depends on the distribution of substance between air and water at equilibrium and how much air that comes into contact with the wastewater. The distribution is described according to Henry's law\textsuperscript{159}, where the concentration in air is in equilibrium with the concentration of the substance in the aqueous phase. The distribution of the substance between water and air depends on how volatile the substance is and is determined or calculated as Henry's constant, which varies widely between different substances. Usually, APIs have very low Henry's constants (<10\textsuperscript{-5} atm, m\textsuperscript{3}/mol) because the substances must remain in the human blood and not be lost with the expired breath\textsuperscript{64}.
Henry's constants for the selected substances in this introduction vary between $5.4 \cdot 10^{-29}$ (Erythromycin) and $1.5 \cdot 10^{-7}$ (Ibuprofen) (atm, m$^3$/mol)$^{60}$. The low values show that the studied APIs are non-volatile and normally do not evaporate into the atmosphere in the WWTP. In summary, no or very little stripping of APIs occurs in wastewater treatment plants$^{64}$.

**Adsorption to sludge**

Adsorption of APIs to different types of sludge in the WWTPs, followed by separation of the sludges from the wastewater, is for some non-biodegradable APIs an important factor for removal.

Adsorption of pharmaceutical residues onto particles occurs partly to primary sludge, in the primary sedimentation, and partly to the activated sludge, in the biological treatment. When adsorption occurs, it is relative fast and supposed to reach equilibrium during the retention time in the respective treatment steps in a sewage treatment plant. Adsorption can normally be described by one of three models: linear (Henry’s), Freundlich or Langmuir isotherms with the corresponding distribution coefficients for linear ($K_d$), Freundlich ($K_f$) and Langmuir ($K_L$) isotherms. In a study of sorption of 75 APIs in municipal wastewater onto primary sludge and secondary sludge, sorption was predominantly best described with linear isotherms, followed by the Freundlich isotherms$^{161}$. This was valid for secondary sludge with either short or long sludge age.

Adsorption with a linear isotherm means that the mass of a substance adsorbed per unit of sludge is linearly proportional to the equilibrium concentration in the solution. The sorption coefficients $K_d$ for a selection of APIs to different types of sludge shows a broad range, Table 5. A high value for the sorption coefficient $K_d$ means high adsorption. For example, ciprofloxacin has ten times higher adsorption to excess sludge, than to primary sludge. Diclofenac has opposite properties, that is, lower adsorption to excess sludge, than to primary sludge.

The most lipophilic substances, with high log $K_{ow}$ (Octanol water distribution constant) values, are hypothesized to adsorb more both onto primary and excess sludge, like e.g. clotrimazole does, Table 5.
Table 5: Sorption coefficients, $K_d$, in primary and excess sludge for a selection of APIs and their octanol water distribution constants, log $K_{ow}^{161,162}$

<table>
<thead>
<tr>
<th>API</th>
<th>$K_d$ primary sludge [L/gSS]</th>
<th>$K_d$ excess sludge [L/gSS]</th>
<th>Log $K_{ow}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>2.6±1.6</td>
<td>26±7.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Clotrimazol</td>
<td>32</td>
<td>34</td>
<td>6.1</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>0.055</td>
<td>0.0024</td>
<td>0.8</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.044</td>
<td>0.02</td>
<td>2.9</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>0.46</td>
<td>0.016</td>
<td>4.3</td>
</tr>
<tr>
<td>Naproxen</td>
<td>0.013</td>
<td></td>
<td>3.2</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>2.5±1.5</td>
<td>37±13</td>
<td>1.0</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>&lt;0.001</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Sulfametoxazol</td>
<td>0.32</td>
<td>0.30</td>
<td>0.5</td>
</tr>
</tbody>
</table>

However, no simple correlation between adsorption and lipophilicity, measured as log $K_{ow}$, can be seen among the selected substances in Table 5, where e.g. ciprofloxacin is readily adsorbed in sediments and onto primary and excess sludge$^{10,163}$. However, replacing log $K_{ow}$ with log $D_{ow}$ (commonly log $D$, the distribution coefficient) the correlation between hydrophobicity i.e. lipophilicity and removal efficiency is improved, since log $D_{ow}$ takes the distribution of ionized and un-ionized molecule into account$^{164}$. Lower pH in the wastewater provides higher adsorption onto sludge for many substances since acidic condition is preferable for the removal of the acidic pharmaceuticals$^{165}$. However, in the relevant pH range, 6–8 for municipal wastewater, no significant effect on the removal via sorption was observed, even though the sorption coefficients, $K_d$s, were significantly different in the pH range 6–8$^{161}$.

**Biochemical transformation – biodegradation**

The biological treatment is the most important treatment step in the existing WWTPs for removal of pharmaceutical residues. For biologically readily degradable APIs, the removal is primarily depending on the sludge age. An important conclusion from the comprehensive EU project Poseidon was that the biological degradation of APIs normally follows a pseudo first order kinetics and depends both on the concentration of the substance and the microbial composition of the sludge, as in turn depends on the sludge age.
Another conclusion was that sludge age, the number of biological reactors in series and the dilution of effluent wastewater with stormwater, are important parameters that affect the removal efficiency in the biological treatment\textsuperscript{162}.

**Impact of biological nitrogen removal (BNR)**

Data from the Swedish WWTPs were also evaluated on the dependence of biological nitrogen removal (BNR) on the removal efficiencies of pharmaceutical residues. One key hypothesis is that high sludge ages in the activated sludge, like the situation in BNR systems, are beneficial for the removal of some APIs. BNR also implies longer hydraulic retention time for the wastewater in the biological treatment, which is beneficial for slowly degradable substances. A comparison of removal efficiencies for a selection of APIs in plants with and without BNR showed that the median removal efficiency was 24\% higher in BNR plants, than in WWTPs without biological nitrogen removal\textsuperscript{63}, Table 6.

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>Removal efficiency in WWTPs with BNR [%]</th>
<th>Number of data pairs (N) used in calculations</th>
<th>Removal efficiency in WWTPs without BNR [%]</th>
<th>Number of data pairs (N) used in calculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>27</td>
<td>12</td>
<td>-45</td>
<td>4</td>
</tr>
<tr>
<td>Citalopram</td>
<td>-48</td>
<td>9</td>
<td>-104</td>
<td>3</td>
</tr>
<tr>
<td>Dextropropoxyphene</td>
<td>-124</td>
<td>11</td>
<td>-149</td>
<td>3</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>11</td>
<td>24</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>Ethinyl estradiol</td>
<td>36</td>
<td>13</td>
<td>74</td>
<td>3</td>
</tr>
<tr>
<td>Furosemide</td>
<td>11</td>
<td>12</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>-13</td>
<td>12</td>
<td>-64</td>
<td>4</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>91</td>
<td>25</td>
<td>77</td>
<td>19</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>58</td>
<td>26</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>-17</td>
<td>17</td>
<td>-41</td>
<td>7</td>
</tr>
<tr>
<td>Naproxen</td>
<td>78</td>
<td>26</td>
<td>54</td>
<td>19</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>-18</td>
<td>12</td>
<td>-16</td>
<td>3</td>
</tr>
<tr>
<td>Sertraline</td>
<td>27</td>
<td>8</td>
<td>-20</td>
<td>2</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>48</td>
<td>18</td>
<td>64</td>
<td>2</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>19</td>
<td>13</td>
<td>-5</td>
<td>4</td>
</tr>
</tbody>
</table>
The negative removal efficiencies reported in Table 6, reflect higher concentrations in the effluent, than in the influent to the WWTPs, which probably is an effect of deconjugation of parent substances in the WWTPs. The amount of data on pharmaceutical residues is limited for Swedish non-BNR plants, in some cases only two to three samples, so the compilation just gives an indication of differences between WWTPs with or without biological nitrogen removal. However, also other studies have shown on the benefit of a higher sludge age of removal of pharmaceuticals. Although with some exceptions, one study showed that for WWTPs operated at SRTs higher than 10 days, low effluent concentrations of many micropollutants was achieved\textsuperscript{140}. The removal efficiency of APIs increased by 40%, from 50% to 70%, at an extremely high sludge age of 75 days in a membrane bio reactor MBR, compared to a conventional activated sludge process, with the normal sludge ages of 5-15 days. A higher sludge age than 75 days did not improved the removal further\textsuperscript{110}. The increased removal efficiency by an increased sludge age is probably caused by a change in the heterotrophic microbial community, rather than an increase in the amount of nitrifying bacteria in the BNR plant\textsuperscript{153}. The relative contribution of the adsorption onto sludge and the biochemical degradation to the total removal differs between APIs, which can be estimated by mass flow analysis over the WWTP\textsuperscript{10,161}.

\textit{Biofilm processes}

Biofilm processes like suspended carriers are hypothesized to achieve higher removal of APIs compared to activate sludge in BNR plants due to a longer retention time for the attached bacteria in biofilm systems\textsuperscript{10,153}. In parallel lab scale batch test, carrier biofilms showed considerably higher removal rates per unit biomass for several pharmaceuticals, such as ketoprofen, and diclofenac, than activated sludges. However, the causes of this are not known\textsuperscript{156}. A comparison in lab and full-scale of carrier biofilm and activated sludge originated from the same WWTP showed that the removal capacity differed for several compounds, with higher removal efficiencies for many micropollutants including diclofenac, mfenamic acid and trimethoprim, what showed significantly higher removal efficiencies with the carrier biofilm compared to different types of activated sludge systems in the examined WWTP\textsuperscript{152}.
Potential of separation processes for removal of pharmaceuticals

Several types of sorbents (activated carbon, minerals and molecular imprinted polymers) have characteristics that make them suitable for removal of APIs\textsuperscript{10,166,167}. While there are relevant sorbents with affinity for most APIs, a common problem with their use is that the full sorption capacity cannot be used, due to fouling of the materials and loss of sorption capacity to irrelevant organic molecules present in the treated wastewater\textsuperscript{168}.

Membrane filtration is a process with separation of particles or solutes over a semi-permeable membrane. In descending order of membrane pore size, the treatment is either called microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) or reverse osmosis (RO). Increasing hydraulic pressure must be applied with the decreasing pore size to separate the substances over the membranes, which demands more electric energy for pumps, but also influence the choice of different types of pumps. MF and UF are applied in membrane bioreactors (MBRs) to separate activated sludge from treated wastewater. The pore sizes of micro- and ultrafilters are much larger than the APIs physical molecular sizes. Tight nanofilters (150 Da) and especially reverse osmosis membranes, have smaller pore sizes than the physical molecular sizes of all APIs available. NF and RO are widely applied in drinking water preparation, to remove salts and small molecules, including trace pollutants from contaminated raw waters and seawater. MF and RO are also used in water reclamation plants, treating effluents from WWTPs though after months in reservoirs\textsuperscript{10,81,169,170}.

1.10.7 Activated Carbon (AC)

Adsorption with activated carbon (AC) is a widespread and general technology to remove organic substances from fluids and gases. The adsorption capacity of activated carbon is high for many organic substances, due to the large porous surface, which varies in the range of 500-1500 m\textsuperscript{2}/g. AC is produced from a variety of raw materials such as coal, anthracite, lignite, wood and coconut shells. The production of commercial AC products involves carbonization and activation. The carbonization process includes drying, heating, pyrolysis and carbonization, within a temperature range of 400–600° in an oxygen-deficient atmosphere. The activation is normally achieved thermally by the use of oxidation gases, such as steam at above
800°C or carbon dioxide at higher temperatures\textsuperscript{43}. During the activation, pores of different sizes are formed, and these are divided into three groups, depending on the sizes of the pore openings: micropores < 2 (3.5) nm, mesopores in the range of 2 (3.5)- 50 nm and macropores >50 nm. Most of the adsorption takes place in the micropores, due to Wan der Waals forces. The values within parentheses relates to the largest distance between two crystalline planes in the microcrystallites, the main constituent in activated carbon\textsuperscript{43,171} and should be a more correct dividing limit value between micropores and mesopores, than the often stated 2 nm. Two main forms of activated carbon are used in water and wastewater treatment: powdered activated carbon (PAC) and granular activated carbon (GAC)\textsuperscript{43}. GAC has a larger particle diameter, ranging from 0.2 to 5 mm compared to PAC with particle diameters < 0.2 mm, typically 0.015–0.025 mm. A large specific surface area of an activated carbon comes from a high distribution of micropores and small mesopores, which was shown to correlate well with a high average removal of organic micropollutants\textsuperscript{172}. A high solute hydrophobicity (log D), was well correlated with higher adsorption of solutes to AC, which has a predominantly hydrophobic surface. In addition, polarizability, aromaticity and the presence of H-bond donor/acceptor groups will influence the adsorption onto activated carbon. These adsorption mechanisms will occur in parallel to different extent, depending of solute and AC characteristics\textsuperscript{168}. Like in membrane processes, remaining bulk organic substances in the wastewater will adsorb onto the AC surface, giving it a negative charge, which favours the adsorption of positively charged substances. In a study of adsorption of PFOS and PFOA, the adsorbed organic bulk material responsible for the negative charge, had a MW < 1000 Da\textsuperscript{173}. Like for the application of oxidation methods for removal of APIs, it is important that the feed of wastewater to the activated carbon contains low concentrations of particles and bulk organic substances. Otherwise, the consumption of activated carbon becomes unnecessarily high and the GAC filters will clog with accumulated particles.
1.10.8 GAC granular activated carbon

Treatment with GAC is normally performed in fixed filter bed units, in smaller applications often with two filters in series, and in larger plants with several filter lines in parallel, to meet the hydraulic capacity demand in the plant. In larger plants, normally only one filter will be installed as a single step, with no succeeding filter. The filter bed is normally 1-3 m deep and must be backwashed regularly to remove accumulated substances that otherwise clog the filter. The filters are filled with a proven GAC product i.e. a product that has shown to adsorb an adequate set of substances listed for removal. GAC products are available in different average particle sizes, in the range of 0.6 to 3.0 mm.

To supervise GAC filter performance, the position of the unsaturated adsorption zone or (average) mass transfer zone (MTZ) is followed to determine when the GAC bed must be exchanged. Organic substances in the municipal wastewater have different concentrations and adsorption characteristics, meaning that they will travel through the GAC bed with different retention times.

Depending on the operational target level of removal efficiency, the exchange of a saturated GAC bed will be done after a varying number of thousand passed bed volumes. The bed volumes (BV) are normally calculated based on the empty filter volume of filled GAC, meaning the volume that the GAC product later will occupy. The hydraulic retention time in the GAC bed is also calculated based on the BV, giving the useful parameter empty bed contact time (EBCT). At the time of GAC exchange, it is only the first of the two filters in series that will be exchanged. The second filter will be put first in series to be fully saturated it before exchange, all for economic reasons.

The spent GAC will preferable be removed and transported to a regeneration plant, where thermal treatment for 30 minutes will volatilize and degrade the adsorbed substances by oxidation at 700-1000°C, followed by reactivation in steam and carbon dioxide. In the regeneration process, 2-10% of the GAC is lost and must be replace by virgin product. The GAC product can be regenerated five times before it is finally wasted and incinerated. The possibility to regenerate used GAC, gives an economic advantage compared to the handling of used PAC, which must be incinerated without regeneration and reuse.171,174,175.
1.10.9 Powdered activated carbon (PAC)

PAC has a smaller particle size than GAC and the average particle sizes of PAC products are in the range of 0.01 to 0.03 mm. Treatment with PAC is either made in existing or additional treatment steps in the WWTPs. The main parts in PAC treatment is a mixing tank for wastewater and PAC slurry, contact tanks, a sedimentation tank and in some application an additional separation unit: a sand filter or an ultrafilter. The contact tanks are completely mixed to ensure effective contact between PAC and organic solutes in the water. The conditions for adsorption of organic solutes onto PAC is closer to steady-state than in GAC applications, due to smaller particle sizes and shorter diffusion pathways. In the sedimentation tank, and in the sand filter, the main fraction of the added PAC is accumulated. To better utilise the dosed PAC, and come closer to adsorption steady-state, the separated PAC in the sedimentation and in the sand filter is recycled back to the contact tanks. This internal recirculation will prolong the solid retention time for PAC in the system and increase the PAC concentration in the contact tanks\(^{176–178}\). The spent, separated PAC is discharged to sludge handling or to a separate handling of PAC, for later incineration. PAC is not recovered or reactivated, due to the handling difficulties and economic considerations. The implementation of PAC in existing treatment trains is easy and has low capital cost\(^{43,174}\). The cost-effective implementation of PAC in existing WWTPs involves the utilisation of the activated sludge stage as contact tank with relatively long retention time for PAC and organic solutes. The spent PAC will mix with the activated sludge and follow the excess sludge to the sludge treatment. In central Europe, with sludge incineration, the adsorbed solutes and PAC itself will be incinerated, together with other organic substances. In Sweden and some other countries, where recycling of anaerobically digested sludge to farmland still is in practice, the PAC dosing to activated sludge would ruin the sludge quality and rule out recirculation of sludge to farmland\(^{10}\).

1.10.10 Nanofiltration and reverse osmosis

The moderate to high pressure-driven tight nanofilters (150 Da) and especially reverse osmosis membranes have been evaluated in pilot scale studies for their potential for removal of APIs in effluents from municipal WWTPs. In a South Korean study, fourteen APIs, six hormones, two antibiotics, three hygiene products and a flame retardant were removed more than 95% by an RO system\(^{179}\). The overall removal efficiencies for
micropollutants, including 11 APIs in a full-scale water recycling plant with MF and RO in Australia, were above 97%, with the exception of 90% removal of bisphenol, with a residual concentration of 0.5 g/L in the final recycled water, which in itself is a serious challenge for process operators\textsuperscript{180}.

The importance of an appropriate choice of NF membrane is demonstrated by a study of the removal of APIs with MWs in the range of 151-791 Da, over an NF membrane with a MWCO of 600 (±200) Da. The removal efficiency varied between 30–90% except, for naproxen (MW 230 Da), which had lower than 10% removal efficiency\textsuperscript{181}. The membrane MWCO must be in the same order as the lowest MW of the APIs expected to be removed from the wastewater. In another study with an NF membrane with MWCO of 150 Da, the removal efficiency of six APIs, all within a relative narrow range of MW (214.6 to 296.1 Da), varied between 68% and 95%\textsuperscript{169}. In a test with two NF membranes in parallel, one tight NF90 (considered to have a MWCO of 200 Da) and one loose HL (considered to have a MWCO of 150-300 Da), the average removal efficiency of eight antibiotics in the MW range of 204-460 Da was 99 and 88% respectively. The loose NF membrane retained smaller antibiotics molecules less effective. The rejection of the examined tight NF membrane was remarkably high\textsuperscript{182}.

By all these examples, it can be concluded that RO, and in some cases NF, can yield very high removal efficiency, 95-99%, of APIs in municipal wastewater. Theoretically an NF with low MWCO should have a higher removal efficiency than an NF with higher MWCO, but in the range 150-300 Da. However, the comparisons above shows the uncertainties for the proportional dependency of MWCO and observed removal efficiency.

The rejection of organic pollutants in NF and RO depends on three major interactions of the organic solute and the membrane: 1) steric hindrance, 2) hydrophobic interactions, and 3) electrostatic interactions. The grade of steric hindrance depends on the molecule size, in comparison to the pore size of the membrane. Hydrophobic interactions mean adsorption of hydrophobic substances onto and into the membrane matrix, which also can facilitate migration of some hydrophobic substance to the permeate, but the influence of hydrophobicity decreases with increasing molecular size in relation to molecular weight cut off (MWCO). Electrostatic interactions are important for rejection of charged organic solutes, due to the negatively charged membrane surface, that repulse negatively charged organic solutes from the membrane surface and prohibit passing to the permeate. Furthermore,
positively charged solutes are enriched close to the membrane surface, facilitating passing of negatively charged molecules, which will lower the rejection\textsuperscript{183--185}.

Nanofiltration uses less tight membranes than reverse osmosis, but as shown above, NF can have a comparable removal efficiencies of APIs, and the use of the less tight NF membranes can reduce the energy costs for pumping by 25--50\%, compared to RO\textsuperscript{186,187}. Still, the energy consumption compared to ozonation is estimated to be 2 and 3 times higher for NF and RO respectively, at high VRFs of 20-60\textsuperscript{187,188}.

\textbf{Retentate handling}

Applying NF and RO for removal of pharmaceuticals in wastewater lead to the question of retentate handling. The membrane technologies concentrate APIs in the retentate, but the APIs will not be eliminated. NF and RO separate much more compounds than pharmaceutical residues, especially inorganic salts when applying RO. NF retains only multivalent inorganic ions, not monovalent inorganic salts, which makes NF a good alternative to RO, as long as the removal of APIs is sufficient\textsuperscript{189}. The salt enriched retentates demand treatment so the effluent standards of APIs for a WWTP can be fulfilled. In water reclamation plants, normally no effluent standards for APIs are set for the wastewater, which lead to a disposal of retentate with enriched API concentrations to a river or the sea. However, the retentate from RO units in wastewater reuse plants, can be treated with advanced oxidation processes (AOPs), in combination with a biologically active sand filters\textsuperscript{190}.

In a WWTP, extended with NF or RO for removal of pharmaceuticals, 90-95\% of the APIs might be concentrated in the retentate, which must be treated to fulfil the effluents standards, therefore a reasonable small volume of retentate is required for an affordable further processing. VRF 20 is an accessible volume reduction factor for NF and RO applied for wastewater treatment, corresponding to 5\% of the inflow ending up in retentate volume. At VRF 60, the retentate volume would correspond to 1.7\% of the inflow to a WWTP. This latter volume of retentate is in the same order of magnitude as the volume of supernatant from digested and dewatered sludge, which constitutes 0.5\% – 1\%, of the incoming flow to the WWTP\textsuperscript{123,191}. 
Potential of chemical oxidation for removal of pharmaceuticals

Advanced oxidation processes (e.g. UV/H$_2$O$_2$, H$_2$O$_2$/O$_3$ and UV/O$_3$) and selective oxidation reagents (O$_3$) have been used to oxidize APIs remaining in traditionally treated wastewater$^{10,192-196}$. The alternative oxidants have different power of oxidation. A comparison, where chlorine gas is used as the reference, shows that ozone has 1.5 times the oxidation power compared with chlorine, Table 7. Hydroxyl radicals have the strongest oxidation power and they have been tested to remove pharmaceutical residues in a system with UV and hydrogen peroxide where they are produced, to a larger extent than by ozonation. Furthermore, ClO$_2$, H$_2$O$_2$, PAA have been tested to remove APIs, see below.

<table>
<thead>
<tr>
<th>Oxidizing species</th>
<th>Relative oxidation power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine dioxide (ClO$_2$)</td>
<td>0.94</td>
</tr>
<tr>
<td>Chlorine (Cl$_2$)</td>
<td>1.00</td>
</tr>
<tr>
<td>Hydrogen peroxide (H$_2$O$_2$)</td>
<td>1.31</td>
</tr>
<tr>
<td>Peracetic acid (PAA)</td>
<td>1.34</td>
</tr>
<tr>
<td>Ozone (O$_3$)</td>
<td>1.52</td>
</tr>
<tr>
<td>Atomic oxygen (-O)</td>
<td>1.78</td>
</tr>
<tr>
<td>Hydroxyl radical (-OH)</td>
<td>2.05</td>
</tr>
</tbody>
</table>

1.10.11 Ozonation - oxidation of substances in wastewater with ozone

Traditionally ozonation, i.e. injection/introduction of ozone (triplet oxygen, O$_3$) into water, has been used in drinking water production for disinfection, taste and odour control and to oxidize iron, magnesium, cyanide, phenol, benzene, chlorophenol, atrazine and other pollutants. Applications on wastewater are also know from water reclamation plants, fed with treated wastewater$^{170,198,199}$.

Ozone is a highly toxic and reactive, with blue colour and intense odour. Ozone is a relatively heavy gas with a density of 2.14 kg/m$^3$, compared to air with a density of 1.29 kg/m$^3$ at T = 0 °C, p = 1 atm. The odour threshold is
about 0.02 ppm and the maximum daily exposure level during 8 h is set to 120 µg/m$^3$, approximately 0.05 ppm in the European union (European Commission, 2016). In Sweden, the maximum daily 8 h exposure level is set to 0.1 ppm and the 15 minute exposure level is set to 0.3 ppm$^{200}$. Acute effects of ozone exposure are headache, dry throat and mucous membranes, and irritation of the nose. Higher concentrations of ozone can also cause delayed lung oedema, cough, choking sensation and other illness. Chronic exposure symptoms are similar to acute exposures, with lung function decrements, asthma, allergies. Tumorigenic, direct and indirect genetic damage have also been found in animal and human tissue studies. However, ozone is also used in medicine by ozone-therapists, despite difficulties in publishing studies in peer-review journals, which have limited the knowledge in official medicine$^{201}$.

Ozone is unstable and must be produced on site. The leading production method is to lead pure oxygen (95-99%), through an ozone generator with a chamber with a high-voltage electrical field, where up to 20% percentage of the oxygen is converted into ozone. In the electrical field, electrical energy excitation of oxygen results in the formation of oxygen radicals, which in turn react with oxygen, forming two ozone molecules from three oxygen molecules. A portion of the formed ozone gas is converted into oxygen again, releasing energy in form of heat, which demands water cooling of the ozone generator. UV or ultraviolet ozone generators are usually not used in demanding ozone applications, due to low production of ozone with relatively low concentrations. The life span of the UV lamps is limited and they must be changed yearly$^{10}$.

Treatment with ozone requires electric energy for on-site oxygen generation, ozone production (including cooling pumps) and for destruction units of residual ozone after treatment.

Ozone reacts with substances in two different ways, by indirect and direct oxidation, leading to substance specific, but mostly unknown oxidation products and the reactions are controlled by different kinetics. The indirect reaction pathway involves radicals to form secondary oxidants such as hydroxyl radicals (·OH) which are formed in the decay of ozone, which is accelerated by initiators, e.g. OH- ions. The ·OH radicals react non-selectively with a number of organic compounds in water and are essential in degradation of persistent substances$^{192,202}$. In the direct oxidation, ozone reacts and splits an unsaturated bond, due to its dipolar structure. Ozone reacts also with aromatic compounds, faster with aromates with electron
supplying substituents, such as the hydroxyl group and amino group, and slower with un-activated aromatic compounds.\textsuperscript{203,204}

Ozone in sufficient quantity and under the right process conditions can oxidize many organic compounds. In WWTPs, with typical water temperature of 10-20°C and pH 7, it is necessary to apply very high doses of ozone to oxidize all substances to carbon dioxide and water (and nitrate, phosphate and sulphate). Often the oxidation stops at the carboxylic acid level. The ozone dose must be selected so especially micropollutants are eliminated or transformed, while preferable the oxidation of bulk organic substances is limited. The ozone treatment step should be installed after the regular biological treatment, where after the concentrations of organic substances are low, to avoid excessive use of ozone. Oxidation of the APIs clofibric acid, ibuprofen and diclofenac occurs within the first few seconds after addition of ozone.\textsuperscript{205} In the diclofenac molecule, ozone attacks the amino group and, in amoxicillin, in addition to the attack on the amino group, benzene rings and sulphur atoms may be possible targets for ozone. The conditions in the water, for example the pH, can influence which functional group that reacts with ozone.\textsuperscript{203} By-products formed after selective reaction with ozone, react further with OH radicals, which causes a chain of reactions. Therefore, only small doses of ozone are usually required for oxidation. The oxidation with ozone, and its reaction with functional groups in the APIs, usually results in the elimination of the pharmacological effect e.g. the estrogenic effect of 17α-ethinyl estradiol is reduced proportionally to the added ozone dose.\textsuperscript{193}

1.10.12 Application of ozonation in water and wastewater treatment

Ozone as a disinfectant of water was documented by de Meritens in 1886 and after pilot plants tests in Germany and a series of discoveries and development of equipment in Germany and France the first full-scale plant for drinking water treatment was taken in operation in 1893 at Oudshoorn in the Netherlands. Many full-scale ozone installations were undertaken until 1914, thereafter the development of inexpensive production of chlorine for chemical warfare opened up for use of chlorine in disinfection of water.\textsuperscript{206}

During the first half of the 20th century, ozonation was used both for disinfection and taste and odour control. In the 1960s, ozonation plants for oxidation of iron and manganese were built, but also ozonation plants for
oxidation of phenolic compounds and pesticides in drinking water. In the 1970s, with the discovery of trihalomethanes (THM) formation by disinfection with chlorine, the market for ozonation plants for disinfection of drinking water increased\textsuperscript{207}. In 2003, water scarcity in Singapore forced recycling of wastewater to produce drinking water for the public. The multibarrier concept for preparing drinking water is named NEWater, where ozonation is used mainly for disinfection of wastewater\textsuperscript{170}.

In 2014, the first municipal full-scale WWTP with ozonation for removal of pharmaceutical residues was taken in operation in Switzerland. The Neugut WWTP in Dübendorf has a design capacity of 150 000 population equivalents (PE) at the present load of 105 000 PE. The overall removal efficiency of five indicator substances was over 80\%, which was also level of the effluent standard\textsuperscript{208}. This first full-scale plant constitutes an important step towards a broader implementation of treatment steps for removal of pharmaceutical residues and it will also serve as a reference plant for other installations, probably worldwide. In 2015, Sweden’s first full-scale plant for removal of pharmaceutical residues was taken in operation in Knivsta (Björlenius, unpublished results). In the latter case, the knowledge that a full-scale implementation of ozonation has been done in Switzerland, confirmed and supported the ongoing work with the ozonation plant in Knivsta.

1.10.13 Ultraviolet Light and Hydrogen Peroxide (UV/H\textsubscript{2}O\textsubscript{2})

The UV/ H\textsubscript{2}O\textsubscript{2}-process is an advanced oxidation process (AOP) and therefore involves production of reactive radicals, particularly the OH radical, favourable for oxidizing organic contaminants in water. Hydroxyl radicals, one of the most powerful oxidants, Table 5, are theoretically easiest produced by cleavage of hydrogen peroxide by ultraviolet radiation. Unfortunately, hydrogen peroxide adsorbs UV-light poorly and therefore the concentration of hydrogen peroxide must be high in the wastewater to generate sufficient levels of hydroxyl radicals. In addition, the UV/H\textsubscript{2}O\textsubscript{2} may involve direct or photosensitized photolysis of the organic contaminants. The UV/H\textsubscript{2}O\textsubscript{2} process is affected by radical scavengers and competitive UV absorbers in the wastewater, limiting the rate of oxidation, e.g. bicarbonate ions are scavenging the hydroxyl radicals and nitrate ions are shielding the UV-radiation\textsuperscript{209,210}. 
UV light is produced by UV lamps mounted in pipes, where wastewater passes. If the wavelength of the produced UV light is within the range of 200 to 280 nm it is called UVC, and it is in this wavelength range, disinfection of bacteria occurs, and hydroxyl radicals are formed, which in turn, degrade organic compounds. In the water and wastewater applications, the produced UV light is either monochromatic with a single wavelength, often 254 nm, or it has a wider spectrum with several wavelengths. UV light alone can cause disinfection, but UV light gives generally a limited removal of APIs in wastewater. However, photolysis by means of UV-light in sunlight can be significance for degradation of some pharmaceuticals in the aquatic environment, e.g. carbamazepine, diclofenac and ketoprofen\textsuperscript{211–216}.

The removal efficiencies for several APIs are high in UV/H\textsubscript{2}O\textsubscript{2} systems. Diclofenac was removed to 95% by UV/H\textsubscript{2}O\textsubscript{2} treatment after 90 minutes treatment. In a parallel setup, ozone removed 95% after 10 minutes retention time\textsuperscript{217}. Paracetamol was mineralized to carbon dioxide, up to 40 % in a UV/H\textsubscript{2}O\textsubscript{2} system in comparing study with an ozonation system, which achieved 30% mineralization to carbon dioxide. Many intermediates and products were identified for both systems\textsuperscript{218}.

The removal efficiency of naproxen in wastewater exceeded 90% after 3 minutes in a UV/H\textsubscript{2}O\textsubscript{2} process\textsuperscript{219}. The dosage of hydrogen peroxide is normally in the range of 3-30 g/m\textsuperscript{3} of wastewater and the demanded contact time ranges from 3 to 90 minutes. The pathway of UV light in wastewater is short, just a few centimetres, depending on the turbidity in wastewater. The design of the UV reactor is therefore important to enable good contact between UV-lamps and the wastewater to get high removal efficiencies of APIs. The UV-radiation doses range from 70 to 400 Wh/m\textsuperscript{3} \textsuperscript{10}.

In summary, UV/H\textsubscript{2}O\textsubscript{2} systems remove many APIs, but the hydraulic retention time is longer, and the energy consumption is higher, than in ozonation systems. The addition of H\textsubscript{2}O\textsubscript{2} and the periodical exchange of UV-lamps increase the cost further. The scaling reduces the UV transfer and too high turbidity is critical for the application of UV/H\textsubscript{2}O\textsubscript{2} in wastewater treatment.
1.10.14 Combination of UV/TiO$_2$

Under UV exposure of a solid phase catalyst, generally titanium dioxide (TiO$_2$). The catalyst adsorbs UV light and hydroxyl radicals are formed from water molecules or hydroxyl groups, adsorbed onto the catalyst surface. The formed radicals react with organic substances by redox reactions$^{220-223}$. One benefit of using TiO$_2$, instead of a continuous dosing of hydrogen peroxide, is the lower running cost for the treatment. UV in combination with titanium dioxide (TiO$_2$), has shown promising results for e.g. for diclofenac, with 95% removal efficiency, also at high diclofenac concentrations$^{224}$. Photocatalysis with TiO$_2$ was more efficient than photolysis alone, in the degradation of 17β-estradiol, estriol and 17α-ethinyl estradiol in water$^{225}$.

1.10.15 Combination of hydrogen peroxide and ozone (H$_2$O$_2$/O$_3$)

The addition of hydrogen peroxide (H$_2$O$_2$) to an ozonation step can enhance the production of OH radicals and consequently increase the potential for a higher removal of APIs. In a lab scale study, the addition of H$_2$O$_2$, up to 1.3 g/m$^3$ wastewater, had a limited impact on the removal efficiency of APIs, but the addition reduced the necessary hydraulic retention time, which is advantageous in full-scale applications. In another study, the elimination rate for cyclophosphamide and the APIs building block quinoxaline-2-carboxylic acid, increased by 26% by an addition of H$_2$O$_2$, corresponding to 2.3 g/m$^3$ wastewater$^{226-228}$.

1.10.16 Chlorine dioxide (ClO$_2$)

Chlorine dioxide is used in drinking water production for disinfection and for taste- and odour control. Typical doses of ClO$_2$ for taste and odour control, or for disinfection, may be in the range of 0.07–2 g/m$^3$ $^{229}$. The disinfection efficiency in water and wastewater is comparable to use of chlorine, but chlorine dioxide is a better alternative, since it limits the formation of organic disinfection by-products e.g. chloramines, and trihalomethanes$^{230}$. However, chlorine dioxide applied in water treatment is converted to chlorite and chlorate, which is inorganic disinfection by-products formed from chlorine dioxide decay$^{229}$. Toxicity of chlorine dioxide, chlorite and chlorate ions to some species e.g. rainbow trout and brown algae has been reported$^{231,232}$.

Chlorine dioxide is a strong oxidant over a broad pH range$^{230}$. Thus, oxidation of APIs with chlorine dioxide has been proposed as an alternative to
ozonation, due to high removal efficiencies at low doses of ClO$_2$ for some APIs, e.g. 17α-ethinyl estradiol, diclofenac, roxithromycin, sulfamethoxazole and mefenamic acid$^{233,234}$. However, clofibric acid and ibuprofen were not removed when wastewater was treated with ClO$_2$ doses up to 20 g/m$^3$. Extended studies showed on a low degree of reactivity using ClO$_2$, with an average removal efficiency of 32% for 56 APIs tested, even when the ClO$_2$ dose was increased to 20 g/m$^3$. APIs with electron-withdrawing functional groups appear to be more resistant to the ClO$_2$ oxidation.

The high removal efficiency for 17α-ethinyl estradiol, at a low dose of chlorine dioxide, was explained by ClO$_2$’s high reactivity to phenolic moieties. Higher removal efficiency of APIs was achieved treating effluent wastewater from a BNR process, holding a relatively low COD concentration, 30 g/m$^3$, than treating effluent from a WWTP without BNR, which produced a wastewater with a COD concentration of 55 g/m$^3$. This shows the importance of a low content of bulk organic substances in the wastewater to be treated, to reduce the consumption of oxidation agents$^{202,235}$. The residual concentrations of ClO$_2$ in wastewater, after different doses of chlorine dioxide, were low, up to an added ClO$_2$ dose of 10 g/m$^3$ $^{235}$. In summary, ClO$_2$ will only be effective to oxidize certain APIs. Reactions with ozone are generally faster and ozone reacts with a larger number of APIs than ClO$_2$ does$^{202,235}$.

1.10.17 Peracetic acid (PAA)

Peracetic acid (PAA) is a strong disinfectant, with bactericidal, viricidal, fungicidal, and sporicidal effects shown in industrial applications, and PAA has been discussed and used in disinfection of wastewater effluents in full-scale$^{236,237}$. PAA for wastewater disinfection is relatively simple to implement, due to low investment costs. Additional advantages are the absence of toxic or mutagenic residuals or by-products, a small dependence on pH and the need for only a short contact time. PAA can be used for disinfection in both primary and secondary effluents and no quenching of PAA residues is required. Disinfectant doses range from 2 to 7 g/m$^3$ PAA in secondary and tertiary effluents, and from 5 to 15 g/m$^3$ PAA in primary effluents$^{238}$. Major disadvantages associated with peracetic acid disinfection are the high running cost and the increase of organic material in the effluent, due to acetic acid, which also facilitates microbial regrowth$^{236,239}$. 
The relatively strong oxidation potential of PAA, slightly higher than hydrogen peroxide, makes it a potential candidate for the removal of pharmaceuticals residues\textsuperscript{233}. Due to a minor risk of explosions in the preparation of PAA on site at WWTPs, the number of reported tests on removal efficiencies are limited. Batch lab tests, on three different biologically treated municipal wastewaters, showed that PAA was not sufficiently efficient to remove the evaluated pharmaceutical residues\textsuperscript{233}. However, diclofenac and mefenamic acid, which were removed most of the tested substances, achieved a removal efficiency of approximately 90\%, at a PAA dose of 25 g/m\textsuperscript{3}. The removal efficiencies for the other substances varied between 10-50\%, at the same PAA dose. The removal was negatively influenced by an increasing COD concentration\textsuperscript{233}, indicating the consumption of PAA to degrade bulk organic substances present in the wastewater. In summary, PAA cannot be considered as a candidate oxidant of pharmaceuticals, but it remains as an interesting disinfectant chemical for wastewater\textsuperscript{233}.

**Technologies in development**

Removal of pharmaceuticals in water and wastewater has met an increased attention since two decades. Many alternative technologies have been proposed. In addition to the processes described in this introduction, e.g. Fenton reactions, membrane distillation and use of enzymes, algae and fungi have been discussed as candidate process or measures for removal of pharmaceuticals. These candidates are either resource demanding, still under development or adaptation to municipal wastewater applications and they are not further discussed in this thesis.

**Comparison of treatment technologies**

The different treatment technologies described previously have their pros and cons. Removal efficiency, total cost, practical applicability and complexity in process control are essential parameters to be evaluated before a treatment technology can be selected. A comparison of the treatment methods discussed above shows that, among the methods with high and broad removal efficiency of pharmaceutical residues, ozonation are the most favorable method. This is a result of the ozonation’s need for a relatively short hydraulic retention time, ozonation provides at least a partial disinfection of the wastewater and it can be implemented at a relatively low cost, Table 8.
Due to the risk for by products by ozonation, activated carbon is added to the group of main candidates of most suitable methods for removal of APIs, in spite the moderately higher costs than for ozonation. Today, the membrane technologies are too costly to be an alternative for implementation in larger scale. The selected main candidates ozonation and treatment with activated carbon have previously been pointed out as potential methods for the removal of APIs, at least for implementation in full-scale WWTPs\textsuperscript{10,122}.

Ozonation and activated carbon can reach high removal efficiencies for many different APIs, at a relatively low total cost, and they can be implemented in existing WWTPs. Still questions of by-products formulation by ozonation and high consumption of activated carbon, due to interferences and site competition on adsorptive surfaces, must be more investigated, but that can be studied in full-scale plants as well. The by-product formation by ozonation can presumably be minimized by dose control and subsequent biofilm processes, treating the ozonated wastewater. The other treatment technologies described above, have either limitation in overall removal efficiencies, high total costs or must be further studied before implementation.
<table>
<thead>
<tr>
<th>Treatment method</th>
<th>Hydraulic retention time (HRT)</th>
<th>Cleaning/ regeneration</th>
<th>By-products</th>
<th>Disinfection</th>
<th>Removal efficiency</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge (CAS)</td>
<td>Long</td>
<td>No</td>
<td>Excess sludge</td>
<td>No</td>
<td>Low to high and narrow</td>
<td>Low</td>
</tr>
<tr>
<td>Granular sludge (AGS)</td>
<td>Moderate</td>
<td>No</td>
<td>Excess sludge</td>
<td>No</td>
<td>Low to high and narrow</td>
<td>Moderate</td>
</tr>
<tr>
<td>Membrane bio reactor (MBR)</td>
<td>Moderate</td>
<td>Chemical cleaning</td>
<td>Excess sludge</td>
<td>No</td>
<td>Low to high and narrow</td>
<td>High</td>
</tr>
<tr>
<td>Biofilm systems</td>
<td>Moderate</td>
<td>No</td>
<td>Excess sludge</td>
<td>No</td>
<td>Low to high and narrow</td>
<td>Moderate</td>
</tr>
<tr>
<td>Activated Carbon (GAC)</td>
<td>Short</td>
<td>Backwash of GAC filters</td>
<td>Spent GAC must be regenerated</td>
<td>No</td>
<td>High and broad</td>
<td>Moderate</td>
</tr>
<tr>
<td>Activated Carbon (PAC)</td>
<td>Short</td>
<td>Backwash of filter units</td>
<td>Spent PAC must be incinerated</td>
<td>No</td>
<td>High and broad</td>
<td>Moderate</td>
</tr>
<tr>
<td>Nanofiltration (NF) &amp; reverse osmosis (RO)</td>
<td>Short</td>
<td>Chemical cleaning</td>
<td>Retentate must be treated</td>
<td>Partly/ Yes</td>
<td>High and broad</td>
<td>Moderate to High</td>
</tr>
<tr>
<td>Ozonation (O₃)</td>
<td>Short</td>
<td>Backwash of sand filters</td>
<td>Partly degraded substances</td>
<td>Partly/ Yes</td>
<td>High and broad</td>
<td>Low to moderate</td>
</tr>
<tr>
<td>UV/H₂O₂</td>
<td>Short</td>
<td>Chemical &amp; mechanical cleaning</td>
<td>Partly degraded substances</td>
<td>Yes</td>
<td>Low to high and narrow</td>
<td>High</td>
</tr>
<tr>
<td>H₂O₂/ O₃</td>
<td>Short</td>
<td>Backwash of sand filters</td>
<td>Partly degraded substances</td>
<td>Yes</td>
<td>High and broad</td>
<td>Moderate to high</td>
</tr>
<tr>
<td>Chlorine dioxide (ClO₂)</td>
<td>Short</td>
<td>No</td>
<td>Partly degraded substances/chlorite &amp; chlorate</td>
<td>Yes</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>Peracetic acid (PAA)</td>
<td>Short</td>
<td>No</td>
<td>No toxic by-products</td>
<td>Yes</td>
<td>Low to high and narrow</td>
<td>Moderate to high</td>
</tr>
</tbody>
</table>
Conclusion
Medicines are produced and consumed to cure or alleviate the symptoms of diseases. The active pharmaceutical ingredients in medicines are administrated at high doses, so the effect concentration of the active substance still can be reached in the body. The pharmaceutical residues are excreted mainly in urine, but also in faeces, which are transported in sewer networks to municipal wastewater treatment plants (WWTPs) or discharged directly into watersheds, if no treatment has been implemented in the area. Many pharmaceuticals are resistant to applied removal technologies\textsuperscript{1,10} and passes therefore through the WWTPs, to be discharged into the receiving waters.

Recent investigations\textsuperscript{240,241} suggest that pharmaceutical residues are accumulating in the aquatic environment, with hitherto unknown half-life. The effects of the discharged pharmaceutical residues from municipal WWTPs are mainly unknown, but it can be assumed that the risk of adverse effects in the environment is relatively high, due to the persistence, toxicity and bioaccumulation of many substances. Receptors in animals, living in the environment and subjected to discharged wastewater, are supposed to act either similar to humans or dissimilar, to rise completely different, but still severe, biological effects\textsuperscript{242}. Since this might be an urgent existing and future problem, there is a need of development and implementation of appropriate technical solutions for WWTPs to remove pharmaceuticals.
2 Aim and strategy

The overall aim of the present work is to provide the appropriate knowledge enabling the WWTPs to remove pharmaceutical residues (APIs), according to the demand of legislation and with a methodology and efficiency according to the present scientific frontline.

The study was disposed into two main parts, formulated as four research questions. In the first part, prevalence and modelling of APIs in the environment was examined to confirm the magnitude of the problem with persistence and spreading of APIs in the environment. In the second part, different advanced treatment technologies to remove APIs from municipal wastewater were evaluated in field studies.

The applied strategies were divided into four areas and they can be summarized as follows:

**Area 1)** Mapping of sources and transfer of APIs into the aquatic environment. (Paper I, II and III)

The consumption patterns of APIs and the concentrations of APIs present in the effluent from major Swedish WWTPs were studied and modelled to describe the present situation with transfer of APIs to the aquatic environment. The prevalence of APIs in the Baltic Sea was mapped by sampling in the sub-basins of the Baltic.

**Area 2)** Do APIs accumulate in the aquatic environment? (Paper I)

The Baltic Sea was chosen as a model environment to study and determine the prevalence of APIs in the aquatic environment, since the Baltic Sea is the receiving waterbody for treated wastewater from many Swedish WWTPs.

The prevalence of APIs was mapped by sampling the Baltic Sea sub-basins and the data were used to model accumulation, half-life and predict future concentrations of a selected target substance i.e. carbamazepine. The model was thereafter used to predict environmental concentrations (PEC) of this substance and later compared them with the measured environmental concentrations (MEC) of carbamazepine.
**Area 3)** Among conventional and advanced treatment systems - is there a technology that can remove APIs in a majority of the WWTPs? (Paper II-V)

The work on removal of APIs was based on finding treatment technologies that work broadly, on as many substances as possible. From a list of potential technologies, the most promising were activated carbon, nanofiltration and ozonation, which were selected based on potential high performance and low to moderate cost. Initial lab tests supported the design and construction of pilot plants, with the most promising technologies. To evaluate the validity of the results, pilot tests were performed at four WWTPs, with different characteristics in wastewater composition and process configuration. The goal for the average removal efficiency of the APIs by the selected technologies was set to 95%. The pharmaceuticals were chosen as representatives for APIs that are persistent, bioaccumulating and toxic.

**Area 4)** Does the present discharge of APIs lead to biological consequences in the aquatic environment? (Paper V)

Ecotoxicity tests were planned to estimate the potential effects of regular and advanced treated wastewater on the aquatic environment. For the selected technologies ozonation and activated carbon, field experiments were conducted to study the effects on rainbow trout of untreated and treated effluents. Selected biomarkers in the fish were used to establish the bioeffects in both rapid and slow responding metabolism of xenobiotics such as APIs.
3 Methodology

This work was based on sampling, results from analysis, data collection and modelling, as well as the designing and construction of two pilot plants, whereof one was equipped with fish tanks for ecotoxicity tests.

Sampling

Grab sampling of surface water from 43 locations in the Baltic Sea and Skagerrak was carried out by the crews on three ships, including the tall ship Briggen Tre Kronor. Samples in the Barents Sea and the Greenland Sea were taken from drilled holes in the sea ice. Samples of the WWTPs effluents and treated wastewater in the mobile pilot plant were continuously collected, using a 16-channel peristaltic pump (Ismatec IP16, Cole-Parmer GmbH, Germany). Grab samples were taken in the nanofiltration/reverse osmosis (NF/RO) plant and some of the permeate samples were pooled to form composite samples of mixed permeate. The samples were stored at 6°C during sampling and frozen at -20°C, before shipping to the analytical laboratory.

Measurements and analysis

3.1.1 Process parameters

Conductivity and pH were measured using a portable multiparameter meter (Orion Star A329, Thermo Scientific). Turbidity was measured using a spectrophotometer with a light detector placed 90° in relation to main light path (Nanocolor VIS, Macherey-Nagel) and oxygen concentration in wastewater was measured with an oxygen meter (HQ30D Portable Multi Meter, HACH). In the mobile pilot plant, the incoming wastewater flow to the individual treatment lines was recorded by water meters, supplied with internal registers and pulse outputs for external reading (Z-System, Qn 1.5, Systeme-sh). The transformed flow values were stored in a logger 2020 system (WebIQAB) together with wastewater temperatures, measured every minute by digital sensors (1-wire PRO, Dallas).
3.1.2 Lab analysis of traditional WWTP parameters

Total organic carbon (TOC) and dissolved organic carbon (DOC) were determined using cuvette tests (LCK 385, Hach Lange) and measured with a Hach Lange DR 3900 spectrophotometer. DOC was analyzed like TOC, but after filtration through 0.45 mm membrane filters (Puradisc AQUA, Whatman). Absorbance measurements at 254 nm (UVA254) were done with a Hitachi U-2900 UV spectrophotometer, using quartz cuvettes with a 50 mm path length (VWR). Suspended solids analysis was performed according to wastewater standards (ESS Method 340.2), using glass-microfiber filters of grade GF/A (Munktell).

3.1.3 Analysis of pharmaceutical residues

The pharmaceutical residues in the wastewater samples were analysed by Umeå University, as a collaboration within the MistraPharma project. The concentrations of 100 target pharmaceuticals were determined using liquid chromatography coupled with mass spectrometry (LC-MS/MS). A triple-stage quadrupole MS/MS TSQ Quantum Ultra EMR (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela LC pump (Thermo Fisher Scientific, San Jose, CA, USA) and a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland) operated using Xcalibur software (Thermo Fisher Scientific, San Jose, CA, USA) was used for the analysis of the water samples. A detailed description of the method is given in a published paper243.

Data collection

Data on consumption of APIs in different countries, counties in Sweden and concentrations in influent and effluent in Swedish WWTPs were collected from on-line data bases, official reports and compilations of available literature data64,65,244–254,254–270.
Calculations and Modelling

3.1.4 Removal efficiency

The removal efficiency for an API \( i \), \( RE_i \), was calculated as

\[
RE_i (\%) = 100 \times \left( \frac{c_i - c_j}{c_i} \right)
\]  

(1)

Where \( c_i \) and \( c_j \) were the compound concentrations in the influent and effluent of a tank, watershed system etc. In the case where influent concentration was above LOQ and the effluent concentration was below LOQ, half the LOQ value was used as \( c_j \) in the calculation of \( RE \).

3.1.5 Modelling of environmental concentrations of APIs

A grey box model for calculation of concentration of an API in the Baltic Sea was developed based on a series or system of mass balances for substances in individual sub basins, where each sub basin was approximated as a completely mixed tank reactor. The mass balances were set up based on general expression of the conservation of mass, equation (2).

\[
\text{Input} + \text{Produced} = \text{Output} + \text{Accumulated}
\]  

(2)

Data used in the model were: 1) sub basins characteristics; geographical position, volume, surface area, air and water temperature, precipitation, evaporation, river discharge and removal efficiency of pharmaceuticals from smaller watersheds and 2) statistics on population size and geographical distribution, specific consumer use of API, excretion rate in humans and average removal efficiency in wastewater treatment plants.

The solutions of the equations for different sub basins were obtained by iteration. A set of yearly mass balance equations were set up for the system of interconnected sub basins and solved by iterations (\( N=20 \)) in MS Excel. The output of the model was time series of annual concentration of an API, in all sub basins.

Wastewater treatment plants selected for pilot tests

Four WWTPs were selected in this study, due to significant differences in connected population, different consumption pattern of pharmaceuticals in the WWTPs catchment area, discrepancies between tertiary treatment processes in the WWTPs, as well as differences regarding process conditions such as hydraulic retention time (HRT), sludge loading rate and sludge age.
The four selected plants were Henriksdal WWTP, Käppala WWTP, Kungsängsverket in Uppsala (Uppsala WWTP) and Kungsängsverket in Västerås (Västerås WWTP). Data on WWTP characteristics and effluent wastewater quality are given in Table 12 in the present investigation.

**Henriksdal WWTP** is the second largest WWTP in Sweden and the largest in the Stockholm region and it treats on average 250,000 m³ wastewater per day, corresponding to 750,000 population equivalents. The wastewater treatment consists of pre-treatment (screening and grit removal), primary sedimentation, biological treatment and sand filtration for removal of particles. The biological treatment is an activated sludge process with pre-denitrification, typical for Swedish WWTPs, with biological nitrogen removal (BNR). Chemical precipitation of phosphorous is done by addition of ferrous sulphate to the primary sedimentation tanks and to the sand filters. The treated wastewater is discharged at 30 m depth, in the inner part of the Stockholm archipelago.

In this study, only nanofiltration was piloted at Henriksdal WWTP.

**Käppala WWTP** is the second largest WWTP in the Stockholm region and the plant treats 149,000 m³ wastewater per day, corresponding to 425,000 population equivalents. The treatment consists of pre-treatment (screening and grit removal), primary sedimentation, biological treatment and sand filtration. Two thirds of the wastewater are treated in a conventional activated sludge pre-denitrification, with chemical pre and post precipitation of phosphorous by addition of ferrous sulfate. One third of the wastewater is treated biologically with the UCT (University of Cape Town) process\(^{271}\), which involves enhanced biological phosphorous and nitrogen removal in activated sludge systems. The biologically treated wastewater is collected and distributed to the final sand filters. The treated wastewater is discharged at 45 m depth, in the inner part of the Stockholm archipelago.

**Kungsängsverket** (Uppsala WWTP) treats 50,000 m³ wastewater per day, corresponding to 148,000 population equivalents. The treatment consists of pre-treatment (screening and grit removal), primary sedimentation, biological treatment and final chemical precipitation of phosphorous by flocculation and lamella separation. Approximately 40% of the wastewater is
treated in a conventional activated sludge pre-denitrification setup, while the remaining 60% is treated using activated sludge in a step-feed pre-denitrification setup. Both pre- and post-precipitation are used to remove phosphorous through addition of ferric chloride. The treated wastewater is discharged into river Fyris.

In Uppsala, the mobile pilot plant was extended with a pretreatment step in the form of two shallow sand filter lines, after the early observations of a fast clogging in the upper surfaces of the GAC filters. The sand filters were installed upstream of the leveling tank in the pilot plant, from which all treatment lines were fed.

Kungsängens WWTP (Västerås WWTP) treats 48 000 m$^3$ wastewater per day, corresponding to 102 000 population equivalents. The treatment consists of pre-treatment (screening and grit removal), primary sedimentation and biological treatment, the latter in the form of a conventional activated sludge, with pre-denitrification setup. Methanol and ethylene glycol are used as carbon sources to improve the nitrogen removal process. Pre-precipitation is used to achieve phosphorous removal through addition of ferrous sulfate. Polymeric coagulants are added to the secondary sedimentation tanks to improve particle separation, before discharge to the effluent channels. The treated wastewater is discharged into lake Mälaren.

The shallow sand filters installed in Uppsala were in operation in Västerås as well.

Design, construction and description of pilot plants

Two different pilot plants were used in the study. The nanofiltration plant was designed and constructed within the Stockholm Water project “Prevalence and removal of pharmaceuticals in wastewater”\textsuperscript{10}, where the author was responsible for the pilot testing and the evaluation of the tests performed within the project. The nanofiltration plant was only operated at Henriksdal WWTP. The mobile pilot plant, with activated carbon, ozonation and biofilm processes, was designed, constructed and operated within the KTH part of the Swedish MistraPharma project. The latter pilot plant was operated at the WWTPs in Käppala, Uppsala and Västerås.
3.1.6 Nanofiltration (NF) pilot plant

The membrane pilot plant unit was designed to work in a semi-continuous mode, with an average hydraulic capacity of 800 L/h. Two NF membranes were arranged in a two-staged array system, Figure 7. The total water volume in the membrane unit was about 200 L, including the work tank volume of 180 L. After the final sand filter treatment in the WWTP, wastewater was conducted to the pilot plant work tank and the flow was controlled with a ball cock. From the work tank, the feed was pumped through a cartridge filter (10 µm mesh), to the high pressure pump. After passing the two membranes, the retentate flow was divided, one part was continuously recycled back to the work tank, through a heat exchanger to keep the temperature in the wastewater at 21°C. The other part was bypassed to the inlet of the high-pressure pump. The applied hydraulic pressure was regulated by a needle valve placed on the bypassing retentate pipe. In order to allow for backflow diffusion, the membrane process was stopped for 20 s every 20 min. The membranes used in this study were two commercial spiral wound thin film nanofilter membranes, with aromatic composite polyamide sheets (ESNA1-LF-4040, Hydranautics). The nominal area of each membrane was about 7.9 m² and the molecular weight cut-off (MWCO) of the membranes was 150 Da. The membranes have a low fouling tendency and were neutral to slightly negatively charged and were chosen due to the relatively low MWCO, which theoretically gives a high retention of nearly all APIs.

![Figure 7. Outline of the nanofiltration pilot plant.](image-url)
3.1.7 Activated carbon, ozonation and biofilm mobile pilot plant

A mobile pilot plant was designed and constructed in-house for application at Käppala, Uppsala and Västerås WWTPs. Treatment tanks, and equipment for sampling and process control, were connected and installed into an insulated, 20-foot shipping container, allowing for operation at outdoor temperatures down to freezing, Figure 8.

![Figure 8. The mobile pilot plant with technology for additional treatment Photo: Berndt Björnenius.](image)

The pilot plant contained eleven treatment lines; three designed for GAC application, another three for PAC, two ozonation lines, two lines using biofilm (MBBR) and finally one line with sand filtration after ozonation. The process tanks were all made of stainless steel, except the contact columns for the ozonation, which were made from PVC pipes. An applied control system served the lines to control inlet pumps, water levels in GAC and sand filters, the ozonation dose, dosing of PAC and of flocculation agent. The control system also stored process data for later evaluation.
An overview of the treatment lines and the hydraulic pathways in the mobile pilot plant is presented in Figure 9.

During operation, effluent wastewater was pumped by a submerged impeller pump, via coarse particle filters (2 mm perforation), to a leveling tank located outside the container. The treatment lines were fed directly or indirectly from the leveling tank by separate screw pumps. GAC filtration was performed in three identical treatment lines, but with filter lines filled with different GAC products. Each one of the GAC lines consisted of two filters (columns) in series. The filters were 2 m height and had an inner diameter of 0.15 m each. The operational volume for each GAC line was approximately 60 L. The filters were filled with 1 m of GAC and operated with down-flow configuration. The water level in the GAC filters was kept constant by a control valve in the bottom of the filter, which was connected to the pilot plant control system. Backwashing of the filters was performed with tap water regularly.

PAC treatment was equally performed using three identical treatment lines. Each line consisted of an initial mixing tank for effluent wastewater and dosed PAC, followed by three sequential aerated contact tanks, a sedimentation tank and a final dual media sand filter. The contact tanks were aerated for mixing purposes. The contact tanks, sedimentation tank and sand filter had an operating volume of 100 L, 180 L and 75 L respectively, making a total operational volume for each PAC line of 360 L, six times the volume in of a GAC line. The sand filter was filled with a 0.33 m bottom layer of sand, 1.2-2 mm particle size (Rådasand AB) and a 0.77 m top layer of crushed, expanded
clay with 2-4 mm particle size (Filtralite MC, Saint Gobain Byggevarer AS).
Recirculation of PAC, from the bottom of the sedimentation tank, back to the first, second or third contact tank was accomplished by an airlift pump.

The design of the two ozonation lines in the pilot plant was based on tests with a lab scale ozonation plant, with a 5 m high contact tank equipped with two alternative devices for dosing of ozone: a) bubble diffusor or b) venturi injector. The lab tests showed that higher removal efficiency was achieved with a venturi injector, than with bubble diffusion for the ozone dosing. Based on this, two ozonation lines were designed, constructed and fitted into a mobile pilot plant, Figure 10, where ozone was produced from oxygen, in an ozone generator and the produced ozone, and the remaining oxygen, was added to the wastewater by a venturi injector.

The ozone was produced by an ozone generator (ICT-10, Ozone Tech Systems, Sweden) supplied with 92-99.5% oxygen and was supplied with water cooling. A venturi injector (Mazzei Injector Company LLC, Bakersfield, CA, USA) was used for the addition of the ozone-containing oxygen into the wastewater. The wastewater was thereafter distributed between ozonation line 1 and line 2.

Ozonation line 1 consisted of three, 5 m high, contact columns (CC1, CC2 and CC3), with an operational volume of 83, 167 and 267 L. The contact columns had a subsequent, 200 L aerated stripping tank, for the removal of potentially remaining residues of free ozone, and finally a sand filter, allowing growth of biofilm, for the potential removal of biodegradable products, released in the ozonation. One of the contact columns was used at a time, or they were coupled in series, to extend the retention time for ozone-wastewater contact.

Alternatively, in line 2, the wastewater passed a pressurized contact tank (CT1) for wastewater containing 300 L, with a height 1.7 m and a diameter of 0.54 m. The wastewater was pressurized by an inlet pump to 1.4 bar absolute pressure. A final 33 L aeration tank was installed for the removal of potential residues of free ozone, from the ozonated wastewater. Oxygen was supplied in gas cylinders (99.5% O₂) or alternatively by an on-site oxygen concentrator (92-96% O₂) (Onyx+, AirSep Corporation, Buffalo, NY, USA). The flow of wastewater through the plant was typically 0.4-1.2 m³/h. An ozone dose between 1 and 20 g/m³ could be added to the wastewater and the dosing was managed from the control system in the mobile pilot plant.
Figure 10: Layout of the ozonation pilot plants. Line 1 has three 5 m high contact column (CC1-CC3) and line 2 has one 1.7 m high contact tank (CT1).

Ten 50 L fish tanks were installed in a rack inside the mobile pilot plant for intermittent ecotoxicity tests using rainbow trout. In the setup, five wastewaters with duplicates could be tested in parallel. Effluent wastewater from the WWTP served as the positive control and tap water as the negative control. The retention time for the wastewater was one hour in each fish tank. Aeration via ceramic air stones supplied the water with sufficient, >95% oxygen saturation.
3.1.8 Operation of the mobile pilot plant at the specific WWTPs

The mobile pilot plant was operated for 57 weeks in total, at three different WWTPs. After commissioning of the mobile pilot plant, evaluation tests with flow proportional water sampling started in October 2013 at Käppala WWTP. Weekly samples were collected from 14 sampling point. 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.

After the first four months of operation, sampling and preliminary evaluation at Käppala WWTP, 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 most obvious observation, showing just after a few days, was that without a final sand filter like at Käppala WWTP, the up time for granular activated carbon filters (GAC) was limited. To prolong the uptime for the GAC-filters, a pretreatment in form of sand filters was built upstream 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 and moved to Kungsängsverket WWTP in Västerås, where continuous operation and factorial experiments were commenced and performed to mid December 2014, when the pilot plant was moved back to Käppala WWTP, where complementary tests were done on pretreatment, control of ozone addition, performance of GAC and powdered activated carbon (PAC), during spring 2015.
4 Result and discussion

To reach the proposed aim, the work has been divided into four strategic areas, stated under chapter 2, “Aim and strategy”. The areas are:

Area 1) Mapping of sources and transfer of APIs into the aquatic environment. (Paper I, II and III)

Area 2) Do APIs accumulate in the aquatic environment? (Paper I)

Area 3) Among conventional and advanced treatment systems - is there a technology that can remove APIs in a majority of the WWTPs? (Paper II-V)

Area 4) Does the present discharge of APIs lead to biological consequences in the aquatic environment? (Paper V)

Each strategic area is treated below and relates mainly to the results presented in the attached papers, but results are also related to other publications.

Area 1) Mapping of sources and transfer of APIs into the aquatic environment.

4.1 Use of pharmaceuticals (Paper I-V)

The specific use of pharmaceuticals was shown to vary between country regions and countries. The use of specific pharmaceuticals also changes with time. The overall tendency is 3% yearly increase of total consumption of medicines, especially of pharmaceuticals related to ageing and chronic diseases, but also an increase due to changes in clinical practice. The studied APIs were selected as preferable being persistent, bioaccumulation and persistent, but also sold in considerable amounts.

4.1.1 Use of carbamazepine in countries in the Baltic Sea catchment area

The specific consumption of carbamazepine [DDD/1000 inhabitants] in the countries in the Baltic Sea catchment area were extracted from different, mainly national, official sources but compilation of available literature data was also performed. In the case of unavailable official data, interpolation and
estimations of similar trends in consumption patterns, as in countries with available data sets, were used to estimate the load of carbamazepine to the Baltic Sea, Table 9.

**Table 9.** Specific consumption in 2013 of carbamazepine in the countries in the Baltic Sea catchment area. The specific consumption is given as daily defined dose per 1000 inhabitants. *Estimated values based on few data and general consumption pattern in Sweden (Paper I).*

<table>
<thead>
<tr>
<th>Country</th>
<th>DDD/1000 inhabitants in 2013</th>
<th>Country</th>
<th>DDD/1000 inhabitants in 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belarus</td>
<td>0.78*</td>
<td>Lithuania</td>
<td>0.98</td>
</tr>
<tr>
<td>Czech Republic</td>
<td>1.47*</td>
<td>Norway</td>
<td>1.37</td>
</tr>
<tr>
<td>Denmark</td>
<td>1.00</td>
<td>Poland</td>
<td>2.4*</td>
</tr>
<tr>
<td>Estonia</td>
<td>2.15</td>
<td>Russia</td>
<td>0.34*</td>
</tr>
<tr>
<td>Finland</td>
<td>1.54</td>
<td>Slovakia</td>
<td>1.23*</td>
</tr>
<tr>
<td>Germany (including former GDR)</td>
<td>1.47</td>
<td>Sweden</td>
<td>1.74</td>
</tr>
<tr>
<td>Latvia</td>
<td>0.9</td>
<td>Ukraine</td>
<td>0.21*</td>
</tr>
</tbody>
</table>

Table 9 shows that the specific consumption of carbamazepine is not uniform in the Baltic Sea catchment area. One explanation can be that alternative APIs to treat epilepsy are in use to different extents in different countries, with the most obvious difference for Ukraine, Russia and Belarus. This is most likely a legacy from the former Soviet Union, where methindione was used to treat epilepsy247.

4.1.2 Use of pharmaceuticals in regions – counties in Sweden

The consumption of pharmaceuticals varies also between regions in a country. In 2013, selecting carbamazepine as an example, consumption in the 22 counties in Sweden, showed a variation from the lowest consumption in the Stockholm County, 1.45 DDD/1000 inhabitants, to the highest consumption in the Jönköping county, where 2.59 DDD/1000 inhabitants were used245.

A comparison of statistics from 2014, for the three counties visited during the tests with the mobile pilot plant, showed discrepancies in consumption of ten APIs in this study, which were quantified in almost all samples from regular effluent from the three WWTPs, Table 10.
Table 10. Specific consumption of ten APIs, quantified in the pilot studies in this study\textsuperscript{245}.

<table>
<thead>
<tr>
<th>Specific consumption of API [DDD/1000 inhabitants, day]</th>
<th>Käppala WWTP</th>
<th>Uppsala WWTP</th>
<th>Västerås WWTP</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>County council</td>
<td>Stockholm (Paper III-V)</td>
<td>Uppsala (Paper III-V)</td>
<td>Västmanland (Paper III-IV)</td>
<td>All</td>
</tr>
<tr>
<td>Atenolol</td>
<td>4.24</td>
<td>10.5</td>
<td>12.4</td>
<td>8.93</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>1.39</td>
<td>1.59</td>
<td>1.97</td>
<td>1.65</td>
</tr>
<tr>
<td>Citalopram</td>
<td>16.9</td>
<td>25.0</td>
<td>23.1</td>
<td>20.9</td>
</tr>
<tr>
<td>Codeine</td>
<td>0.13</td>
<td>0.13</td>
<td>0.24</td>
<td>0.15</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>3.88</td>
<td>4.26</td>
<td>5.39</td>
<td>5.80</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>24.0</td>
<td>23.4</td>
<td>22.4</td>
<td>23.3</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>6.51</td>
<td>10.4</td>
<td>9.26</td>
<td>7.85</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>2.71</td>
<td>2.86</td>
<td>4.37</td>
<td>3.21</td>
</tr>
<tr>
<td>Tramadol</td>
<td>2.49</td>
<td>4.15</td>
<td>5.47</td>
<td>3.96</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.13</td>
<td>0.12</td>
<td>0.14</td>
<td>0.19</td>
</tr>
<tr>
<td>Σ of 10 APIs</td>
<td>62.3</td>
<td>82.4</td>
<td>84.8</td>
<td>75.9</td>
</tr>
</tbody>
</table>

[DDD/1000 inhabitants]

The consumption of APIs can be imagined as varying within and between cities, due to the demographic situation and maybe also due to the doctors’ customs of prescribing different APIs, especially when alternative APIs are available.

The most quotable differences in consumption between counties, were for atenolol and citalopram. In general, the prescriptions of the selected APIs were highest in Västerås, followed by Uppsala and lastly Käppala. This order corresponds also to the concentrations of APIs in the effluent from the three WWTPs (Paper III, Table 2). In relative numbers, the specific consumption of the sum of the ten selected APIs was 32% higher in Uppsala WWTP catchment area and 36% higher in Västerås WWTP catchment area, than in the Käppala WWTP catchment area. The different consumption patterns influenced the
loads of APIs on the WWTPs and thereby were the concentration of APIs in influent and effluent wastewater influenced. The concentrations of APIs in wastewaters can also have been influenced by the amount of stormwater in the influent to the WWTP, wastewater temperature, process configuration and conditions at the WWTPs, including hydraulic retention time and sludge age in the biological treatment.

4.1.3 Long-term variations in consumption of pharmaceuticals (Paper I)

After the market introduction of a pharmaceutical, the consumption of the pharmaceutical will increase. The long-term development in consumption pattern depends on many factors such as marketing, medical evaluation of effects, new medical treatment areas for the API etc. In the modelling of the concentrations of carbamazepine in the Baltic Sea, the access to long-term consumption data were essential. Carbamazepine was introduced to the market in Switzerland in 1962 and approved as an anticonvulsant in UK in 1965\textsuperscript{246}. The consumption of carbamazepine in four countries in the Baltic Sea catchment area developed differently during the years, Figure 11.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{carbamazepine_consumption.png}
\caption{Specific consumption of carbamazepine in 1975-2013 in four countries in the catchment area of the Baltic Sea (Paper I).}
\end{figure}
The consumption patterns were relatively similar in Sweden and Denmark during the first decade after 1975, which was the starting year of the simulations in Paper I. Then, the specific consumption increased and was maintained at higher levels in Sweden. During the last decade, a similar annual decrease in consumption of carbamazepine was observed in Sweden and Denmark. In Germany, the initially low levels of consumption, might be an effect on the low consumption in former GDR. A similar explanation can probably be found for Estonia, being a former Soviet Union republic, where methindione was used to treat epilepsy. In summary, the tendency is a general decrease in specific consumption of carbamazepine. Alternative APIs have partly and successively substituted carbamazepine in the treatment of epilepsy. The awareness of non-steady state conditions for the specific consumption of pharmaceuticals, is crucial in the long-term modelling of environmental concentrations of pharmaceutical residues.

However, the total specific consumption of all pharmaceuticals, calculated as DDD/1 000 inhabitants, is steadily increasing in many countries. During the period 2000-2015, the average specific consumption of pharmaceuticals in nine ATC groups increased linearly 2.9% per year ($R^2=0.99$) in 14 countries in the OECD (Australia, Belgium, Czech Republic, Denmark, Estonia, Finland, Germany, Hungary, Iceland, Italy, Norway, Portugal, Slovak Republic and Sweden). The ATC groups in the evaluation were: A-Alimentary tract and metabolism, B-Blood and blood forming organs, C-Cardiovascular system, G-Genito urinary system and sex hormones, H-Systemic hormonal preparations, excluding sex hormones and insulins, J-Anti-infectives for systemic use, M-Musculo-skeletal system, N-Nervous system and R-Respiratory system.

The increased consumption will most likely lead to higher concentrations of pharmaceuticals in the environment, above all in aquatic systems, where they have been found globally, often in combination with low removal efficiency in wastewater treatment plants (WWTPs) or a complete absence of WWTPs.
Area 2) Do APIs accumulate in the aquatic environment?

4.2 Pharmaceuticals in the environment (Paper I)

A sampling campaign of the Baltic Sea water was initiated and planned to study the prevalence and mitigation of pharmaceutical residues in the aquatic environment. 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, instead of being accumulated in the environment.

4.2.1 Measured environmental concentrations (MECs)

Since the Baltic Sea is vulnerable to anthropogenic activities, due to a long turnover time and a sensitive ecosystem in the brackish water\textsuperscript{274-275}, it is a suitable location for environmental studies. Furthermore, it is the receiving water body for many Swedish WWTPs.

A sampling campaign, all over the Baltic Sea, was initiated to study the prevalence and persistence of pharmaceutical residues in larger water bodies, Figure 12, and the sampling was designed to cover as much as possible of both coastal and offshore locations. The potential accumulation of pharmaceutical residues can lead to successively higher concentration, which finally reach effect concentrations in organisms in the water. The sampling points were chosen to cover all sub-basins in the Baltic Sea, to provide environmental concentrations, also for modelling purposes later on.

The results from the chemical analysis displayed the range of quantified environmental concentrations of a selection of 93 pharmaceuticals, in 43 locations in the Baltic Sea and Skagerrak area, Figure 13.

Thirty-nine of the 93 pharmaceuticals were detected in at least one sample, with concentrations ranging between 0.01 and 80 ng/L. In general, coastal locations had higher concentrations of APIs, than offshore locations and the highest concentrations were found outside major cities. The anti-epileptic drug carbamazepine was widespread, both in coastal and offshore seawaters (present in 37 of 43 samples) and is thus a good indicator substance for anthropogenic impact in water. The median concentration of carbamazepine was 2.6 ng/L in the Baltic Sea, which was lower than previous reported median, 22 ng/L, but the latter was based on samples from a limited area of the Baltic Sea, namely the coastal areas of Northern Germany\textsuperscript{240}. 
Results and discussion

Figure 12: Baltic Sea with sampling points and main sub basins: BB=Bothnian Bay, BS=Bothnian Sea, GF=Gulf of Finland, GR=Gulf of Riga, BP=Baltic Proper, DS=Danish Straits, KT=Kattegat and SK=Skagerrak. Two samples, 44 and 45, were taken at Svalbard according to the pasted map in the upper right corner. Values of the environmental concentrations are presented in the supplementary material to paper I.

Figure 13: Minimum, median and maximum concentrations (denoted as vertical bars) of quantified APIs, sorted in declining frequency [%].
4.2.2 Prediction of environmental concentration of pharmaceutical residues – Example carbamazepine

Prediction of environmental concentrations of chemicals is valuable in the risk assessment of the use of chemicals and the discharge of chemical residues to the environment. It is also valuable in the work to identify parameters essential for the spreading of chemicals. Furthermore, prediction of environmental concentrations can be used in the prioritizing work of regions relevant for implementation of advanced treatment at WWTPs or at industries.

In this study, a mathematical model was set up to predict concentrations of pharmaceuticals in the sub basins of the Baltic Sea and the persistent and widely used carbamazepine was selected as model substance in the simulations. The modelling included input data from all countries, not only Sweden, to predict environmental concentrations, in particular concentrations of carbamazepine.

The grey box model consisted of a system of mass balances for a single substance, distributed to sub-basins, where each sub-basin was approximated as a completely mixed tank reactor. The mass balances were set up based on the expression for the conservation of mass, equation (2), also stated in the methodology chapter, but is repeated below.

\[ \text{Input} + \text{Produced} = \text{Output} + \text{Accumulated} \quad (2) \]

APIs were not produced in the sub-basins, except in the case if the parent substance was reformed from a conjugated API. Many APIs were removed to different extents in the sub-basins, giving a negative value to the production term. A graphical representation of a mass balance for sub-basin \( i \), is shown in Figure 14.
The system of mass balances enclosed the Baltic Sea and was divided into seven main sub-basins, plus, the to the Baltic Sea adjacent Atlantic sub-basin, Skagerrak, Table 11. Flows of water, originated from precipitation, evaporation and rivers, and mass flows of substances, including discharges of APIs from sewers and WWTP effluents were calculated.

A set of equations were set up for the system of sub-basins and solved iteratively. The input data into the model were sub-basin characteristics and statistics on national population size and geographical distribution in each sub-basin, use of API, excretion rate of humans and average removal in wastewater treatment plants. The output from the model was a collection of time series of annual concentrations of a substance, in all separate sub-basins.

The predicted concentrations of carbamazepine ranged from 0.51 to 2.5 ng/L in the sub-basins, and the corresponding measured concentrations, amounted to 0.57-3.2 ng/L, depending on sub basin location, Table 11. An analysis of the mean absolute errors (MAEs) for the predictive values showed that the data series, applied for the parameters in the model, seemed to be acceptable, the mean absolute error for the predictive values was 0.43 ng/L, which corresponded to 23% of the average measured concentration of carbamazepine in the Baltic Sea waters.
The average concentration of carbamazepine in the Baltic Sea was predicted to be 1.8 ng/L in 2013, compared to the average measured environmental concentration of 1.9 ng/L, which corresponded to an accumulated mass of carbamazepine in the Baltic Sea of 55.6 metric tons. A simulation, with help of the model, was performed to predict future annual concentrations of carbamazepine in the sub-basins of the Baltic Sea. A scenario with a complete stop in consumption of carbamazepine, showed the long response time, a matter of decades, for the total removal of accumulated carbamazepine in the Baltic Sea, Figure 15.

The predicted concentrations of carbamazepine ranged from 0.51 to 2.5 ng/L in the sub-basins, and the corresponding measured concentrations, amounted to 0.57-3.2 ng/L, depending on sub basin location, Table 11. An analysis of the mean absolute errors (MAEs) for the predictive values showed that the data series, applied for the parameters in the model, seemed to be acceptable, the mean absolute error for the predictive values was 0.43 ng/L which corresponded to 23% of the average measured concentration of carbamazepine in the Baltic Sea waters.
Figure 15. Simulated concentration of carbamazepine in the Baltic Sea and sub basins in the past and in the future after a hypothetical case with a complete stop of consumption in 2014, indicated with a vertical dashed line. Highest concentration in year 2013 was predicted to be present in Baltic Proper (x) followed by Gulf of Riga (+), Gulf of Finland (- - -), Danish Straits (●), Bothnian Sea (- - -), Bothnian Bay (-), Kattegat (▲) and Skagerrak (♦) in descending order. The average annual concentration in the Baltic Sea (■) is based on sub basin volumes times predicted concentration divided by total volume in Baltic Sea and Skagerrak.

The simulation clearly showed that carbamazepine will be present in the Baltic Sea decades after a stop in use. The average flushing and degradation half-time for carbamazepine was ten years. It is important to keep in mind the long response time in the environment, when measures or regulations are discussed to phase out persistent chemical substances – a complete stop in use will not immediately result in zero concentrations in the environment.
4.2.3 Half-life of carbamazepine (Paper I)

Based on removal efficiencies of carbamazepine in surface water, which were calculated from literature data and samples taken in this study, an estimation of the average removal efficiency for one year, and the corresponding removal rate were calculated. An overall removal efficiency of 17.3% per year was achieved from measured and calculated data, corresponding to a half-life, $t_{1/2}$, of 3.54 years, or nearly 1300 days, at 10°C. Assuming a 1st order kinetics for the degradation of carbamazepine, the average removal rate, $r$, was $6.2 \times 10^{-9}$ s$^{-1}$, calculated according to $r = \ln(2) / t_{1/2}$. The persistence of carbamazepine makes it thus a good indicator of wastewater intrusion in natural waters.

The half-life for carbamazepine in water, previously reported in two separate studies, was much shorter: 63 days$^{276}$ and 38 days in constant sun light$^{271}$, although they incorporated flushing rate and had a constant sun light in a laboratory environment respectively. In the second study, the water depth was 2.1 cm, compared with the average depth of 55 m in the Baltic Sea. The intensity of solar UV-light decreases with water depth, e.g. at 2 m, the remaining UV intensity at $\lambda=325$ nm is <1% to 55%, depending of the amount of particles, algae etc. in the water. At 10 m depth, the remaining UV intensity is <2%$^{277-279}$. In a recent study that used biodegradation models, the half-live time of carbamazepine in water was estimated to be 201 days$^{280}$. This demonstrates clearly, independent of used model, that slowly degradable substances can generate at least long-term esthetic, or worse, problems in the aquatic environment.

4.3 Pharmaceuticals in wastewater effluents (Paper II-V)

The WWTPs, where the pilot tests were done in this study, were selected to represent different geographical regions, process layouts and treatment performance, which might influence the wastewater matrix and the composition of the treated wastewater. Evaluation of the characteristics of the WWTPs showed discrepancies in all evaluated parameters (Table 12).
**Table 12.** Characterization of the WWTPs during the studies in this thesis, paper II-V.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Henriksdal WWTP (Paper II)</th>
<th>Käppala WWTP (Paper III-V)</th>
<th>Uppsala WWTP (Paper III-V)</th>
<th>Västerås WWTP (Paper III-IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connected population [pe]</td>
<td>750 000</td>
<td>425 000</td>
<td>148 000</td>
<td>102 000</td>
</tr>
<tr>
<td>Specific wastewater flow [L/pe,d]</td>
<td>333</td>
<td>390</td>
<td>305</td>
<td>471</td>
</tr>
<tr>
<td>Temperature [°C]</td>
<td>15</td>
<td>12</td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Sludge age [d]</td>
<td>16</td>
<td>16</td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Hydraulic retention time (HRT) [h]</td>
<td>29</td>
<td>36</td>
<td>38</td>
<td>14</td>
</tr>
<tr>
<td>Suspended solids in effluent [mg/L]</td>
<td>2.8</td>
<td>0.6</td>
<td>4.6</td>
<td>3.7</td>
</tr>
<tr>
<td>DOC in effluent [mg/L]</td>
<td>6.8</td>
<td>9.3</td>
<td>9.0</td>
<td>9.4</td>
</tr>
<tr>
<td>Dilution factor to recipient</td>
<td>68x</td>
<td>116x</td>
<td>20x</td>
<td>12x</td>
</tr>
</tbody>
</table>

The mapping of the concentrations of APIs in effluent wastewater, from the examined WWTPs, was performed to describe and understand how much pharmaceutical residues, that regular WWTPs release to the aquatic environment after treatment. The results of the mapping were also used as part of the characterization of the selected WWTPs, but mainly as reference values in the evaluation of additional treatment technologies for removal of pharmaceutical residues. Effluent concentrations of a selection of APIs, that were frequently quantified in the effluent from the four WWTPs, were compared with the critical environmental concentration (CEC) for the APIs, Table 13. The CECs are the levels of environment concentrations of different APIs, which will bioconcentrate in fish, so the plasma concentration of APIs in exposed fish will be equal to the human therapeutic plasma concentration. Data on concentrations of other APIs are available in paper II-V.
Table 13. Effluent concentrations, critical environmental concentrations and proposed environmental quality standards of chronic toxicity for freshwater in EU\textsuperscript{134} and Switzerland (CH)\textsuperscript{282} of ten APIs from the four WWTPs with pilot tests.

<table>
<thead>
<tr>
<th>Concentration [ng/L]</th>
<th>Henriksdal WWTP (Paper II)</th>
<th>Käppala WWTP (Paper III-V)</th>
<th>Uppsala WWTP (Paper III-V)</th>
<th>Västerås WWTP (Paper III-IV)</th>
<th>Critical environmental Concentration (CEC)\textsuperscript{281}</th>
<th>EQS fresh water CH (EU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>487</td>
<td>250</td>
<td>220</td>
<td>729</td>
<td>792 000</td>
<td>150 000</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>387</td>
<td>221</td>
<td>524</td>
<td>202</td>
<td>346 000</td>
<td>2 000</td>
</tr>
<tr>
<td>Citalopram</td>
<td>220</td>
<td>92</td>
<td>357</td>
<td>206</td>
<td>141</td>
<td>n.d.</td>
</tr>
<tr>
<td>Codeine</td>
<td>57</td>
<td>85</td>
<td>74</td>
<td>498</td>
<td>26 620</td>
<td>n.d.</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>183</td>
<td>287</td>
<td>200</td>
<td>690</td>
<td>4 560</td>
<td>50 (100)</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>1 330</td>
<td>1 203</td>
<td>1 095</td>
<td>684</td>
<td>15 400</td>
<td>8 600</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>61</td>
<td>119</td>
<td>189</td>
<td>120</td>
<td>14 000</td>
<td>n.d.</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>325</td>
<td>178</td>
<td>330</td>
<td>741</td>
<td>30 700</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tramadol</td>
<td>553</td>
<td>258</td>
<td>563</td>
<td>824</td>
<td>4 800</td>
<td>n.d.</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>84</td>
<td>22</td>
<td>77</td>
<td>62</td>
<td>3 300 000</td>
<td>120 000</td>
</tr>
</tbody>
</table>

The concentrations in the effluent varied a lot for the different WWTPs. Several explanations are plausible: 1) At relatively short sludge age, like in Västerås, removal efficiencies of slowly degradable APIs are lower\textsuperscript{140}. 2) Higher specific flow of wastewater, mainly due to stormwater intrusion, will dilute the influent and thereby lower the concentrations of pollutants. 3) Variation in the inhabitants use of API will influence the concentrations. 4) A higher wastewater temperature will increase the removal rate, which will lower the concentrations of degradable APIs\textsuperscript{283}. 5) A long HRT will promote the removal efficiency, due longer time for degradation to APIs, especially if the biological treatment has a long HRT.

The concentrations of APIs in the effluent from the WWTPs were diluted by natural inflows, e.g. surface run-off, in the receiving waters. The average dilution factors in the receiving waters were for Henriksdal WWTP: 68x, Käppala WWTP: 116x, Uppsala WWTP: 20x and Västerås WWTP: 12x, during the year when the pilot tests were done, Table 13.
The dilution factors were sufficiently high to dilute all effluents, even the concentrations of the most critical API in this list – citalopram – were lower in the receiving water, than the predicted critical environmental concentration (CEC), based on bioaccumulation\textsuperscript{281}. The safety factor between critical environmental concentration and calculated environmental concentration was less than 10 for citalopram in the receiving water in Uppsala and Västerås. The concentrations of diclofenac in the effluents were higher than the proposed environmental quality standards (EQS). However, thanks to the dilution, diclofenac concentrations in the receiving waters were lower than the EQS for fresh water.

In the WWTPs, the concentrations in the effluents indicated different prescription patterns between cities and municipalities, where Västerås probably had the highest prescription, and the municipalities connected to Käppala WWTP, had the lowest prescription. The dilution in the receiving water lowered the concentrations of all APIs, below both the critical environmental concentrations and the proposed environmental quality standards (EQS) for the APIs, which indicates that the discharged pharmaceutical residues will not influence fish in the receiving waters by potential bioaccumulation.

To summarize the observations so far in this study, prescription pattern and the configuration of WWTPs, influenced the concentrations and mass flows of APIs into the aquatic environment. Based on the measured environmental concentrations and performed calculations for the Baltic Sea, the half-life of the chemical substance differed between APIs and was as long as several years, as for carbamazepine. A consequence of the slow degradation of hydrophilic, or dissolved APIs, is that they can pass the WWTPs and they can accumulate in water bodies. Carbamazepine was found in offshore waters all over the Baltic Sea. However, the concentrations of the examined APIs were lower in the specific aquatic environment, than the corresponding critical concentration for effects of bioaccumulation in fish. In receiving waters with lower dilution and higher concentrations of APIs in the effluent from WWTPs, critical environmental concentrations can likely be reach for some APIs.
Area 3) Among conventional and advanced treatment systems - is there a technology that can remove APIs in a majority of the WWTPs?

4.4 Removal of pharmaceutical residues by nanofiltration (Paper II)

The pilot plant for nanofiltration (NF) was configured to treat effluent wastewater from Henriksdal WWTP in Stockholm. The NF removed solutes and ions of a certain size from wastewater by an applied pressure over a semipermeable membrane. The treated wastewater was discharged as a permeate from the nanofiltration, while the separated substances were accumulated in the retentate, which therefore must be further treated or disposed. Most APIs have relatively low molecule weights, typically 150-900 g/mol. Initially, pilot tests were designed to evaluate the removal efficiencies of a selection of APIs, abounded in the effluent of Henriksdal WWTP. A multivariate model was built to identify the most descriptive parameters for the removal of APIs.

During the pilot tests, 32 of the 95 APIs analyzed were quantified in the regular effluent from Henriksdal WWTP, which is equipped with sand filters as the last treatment step. The results from the chemical analysis of the nanofilter permeate were used to calculate the removal efficiencies of different APIs, which varied between 38-99%, with a median removal of 90% of the 32 APIs. The total volume reduction factor (VRF) was 20 during the tests, which implies that the treated effluent from Henriksdal WWTP was split into 95% permeate and 5% retentate, whereof the latter must be further treated. The project aim of 95% removal of APIs was not fulfilled with the selected nanofilter membranes, but still the removal efficiencies were high. The NF membranes were selected based on an offered membrane molecular weight cut-off (MWCO), specified to be 150 g/mol, which was lower than the lowest molecular weight (MW) of the selected APIs in the present study. A comparison of the removal efficiencies achieved versus MW, showed that the cut-off for 90% removal for an individual API was approximately 300 g/mol, instead of the offered 150 g/mol, Figure 16. To reach 95% removal of any individual API, the MW of the API must exceed 420 g/mol in this study. The observed lower removal of the smallest APIs, with MWs in the interval 150-300 g/mol, is thus a membrane problem.
Results and discussion

Figure 16: Removal of 32 pharmaceuticals vs. molecular weight (MW) in the nanofiltration studies.

To better understand and predict the removal of APIs in nanofiltration, a model was developed with multivariate analysis methodology, to predict the rejection for a nanofiltration plant, treating effluent from full-scale WWTPs. Many of the studies previously reported on the removal of micropollutants by nanofiltration, were performed in a laboratory environment. By these lab tests, virgin membranes and spiked pure water were used, to avoid interference of the wastewater matrix in the evaluation.

To model the rejection of APIs by nanofiltration, Principle Components Analysis (PCA) and Partial Least Squares Projection of Latent Structures Analysis (PLS) were used. Initially, PCA was used to give an overview of the data, check for outliers and discover correlations between the variables. In this process, four APIs were excluded from the development of the model, being significantly different due to large size or small size in combination with large polar water accessible surface areas compared to the other APIs. However, the four APIs were included in the latter testing of the models.

In the modelling, 59 physiochemical properties of the pharmaceutical residuals present in the treated wastewater were initially used as variables (Paper II – Table 3 in supplementary material). Examples of the molecular properties were molecular size and shape, electrostatic properties, polarity,
hydrophobicity and presence of different functional groups. To distinguish between different physical characteristics, ratios between different variables were also included. Data for the chemical and physical parameters were collected from open databases such as ChemAxon and Chemspider.

In the PLS modelling, the initial 59 variables were reduced to four by successively selecting the best representing variable for similar properties in combination with the relative importance and relationship in the PCA modelling, equation (3). Models were set up and developed for each test and for every VRF in the range from 2 to 20, using all data, as well as a grouping of data into a training set and a test set, Figure 17.

![Figure 17: Regression lines for developed PLS models.](image)

PLS models based on the same VRF, showed very good similarity. However, the accuracy of the models differed for different VRFs. Models based on VRF 10 (ModA:10 and ModB:10 in Figure 17) showed the best conformity to the observed rejections. The rejection was found to be best described by equation (3):

\[
\text{Rejection} = \alpha + \beta \times \text{polarizability} + \gamma \times \text{globarity} + \delta \times \text{phob/polar} + \varepsilon \times \text{charge} \quad (3)
\]

Where \(\alpha\), \(\beta\), \(\gamma\), \(\delta\) and \(\varepsilon\) were plant specific constants and \text{phob/polar} was the ratio between hydrophobic and polar accessible surface area of the molecule. The coefficients in the model were dependent on wastewater composition,
wastewater treatment, water recovery, membrane type and brand, etc. The model was tested on previously reported data from pilot tests with spiked tapwater, since no data were found from tests with regular wastewater. The training sets and validation sets were used to calibrate and simulate rejection of different substances. The simulations showed that the proposed modelling approach can be used, also for other studies, to simulate rejection of substances by nanofiltration. The rejection model was valid in a range of the molecular weights MW, around the molecular weight cut-off value (MWCO). For higher MW, preliminary 2-4 times the MWCO, the clearly determining factor for rejection was the molecule size. The model is recommended as being a practical tool for operators of WWTPs and wastewater reuse facilities, to estimate the rejection of emerging organic compounds, based on their physiochemical characteristics. The influence of the variables varied between the VRF’s and the model coefficients must be developed for each specific WWTP, or water reuse facility, and should be checked up seasonally or yearly.

At low VRFs, removal efficiencies for negatively charged APIs were higher than at high VRFs. The repulsion of negatively charge APIs comes most probably from the typically negatively charged membranes.

In summary, the nanofiltration pilot plant gave high removal efficiencies of APIs, but the achieved 90% average removal efficiency could most probably have been higher if the delivered nanomembrane had met the manufacturer specified value of 150 g/mol for molecular weight cut-off (MWCO). The membrane was shown to have an actual MWCO value of 300 g/mol. The higher MWCO value of the membrane resulted in poor removal of the smallest APIs, having MWs in the range of 150-300 g/mol. The developed model for predicting the rejection of APIs can be a useful tool for operators of a treatment plant, but also in the estimation of removal efficiencies of not analyzed substances. However, the model coefficients must be determined for the individual WWTP, since they are site specific.

4.5 Treatment with GAC and PAC (Paper III and IV)

The aim of the pilot tests was to achieve 95% removal of a selected set of 22 APIs, frequently occurring in Swedish municipal wastewater. This degree of removal was chosen to have a safety margin to critical environmental concentrations (CECs) and future effluent quality standards (EQSs). The 22 APIs were selected after mapping effluent wastewater, among 100 preselected bioactive APIs. The selection criterium was presence of the substance in more than 50% of the samples.
Two GAC units and two PAC units were operated in parallel in the mobile pilot plant, consecutively at three WWTPs. The plants were selected to facilitate evaluation of site-specific conditions, especially wastewater characteristics and plant configuration. Ozonation was operated in parallel with the GAC and PAC units (partly described in paper V). Each treatment line was designed for a hydraulic flow of 100 L/h. The GAC lines consisted of two filter filters in series, with a valve arrangement to allow backwashing and change of filter order, after saturation of the first filter. The HRT, or more specifically the EBCT, was 20 minutes per GAC line. Including the water column above and below each filter, the total HRT in each GAC line was 36 minutes during operation. The corresponding HRTs in the PAC lines, including sedimentation tank and sand filter were 3.5 h, with 60 minutes contact time in all three contact tanks together, Figure 18.

![Figure 18. Schematics of the GAC and PAC treatment systems. The GAC system is composed of two filter columns in series using down-flow operation. The PAC system is composed of an initial mixing tank (denoted "M"), three sequential contact tanks, a sedimentation tank and a sand filter. Sampling points are symbolized "S". R1, R2 and R3 indicates the different recirculation pipes in the PAC system, while RX symbolizes an operation without recirculation of settled PAC from the sedimentation tank.]

4.5.1 Selection of GAC and PAC products

A set of 14 activated carbon products was initially selected, based on a broad range of adsorption specific properties, e.g. specific surface area, iodine number and particle size. Among the 14 products, in total eight GAC and PAC products were selected for tests in pilot scale.

Five GAC products were selected for evaluation in pilot scale, based on a previously performed in-house tests10, but also based on carbon properties and prequalifying recommendations from manufacturers. Initially, eight prequalified PAC products were tested in bench scale and evaluated on the
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removal efficiencies of all quantified APIs, among the list of 100 preselected bioactive APIs\textsuperscript{10,243,281}. Three PAC products were selected for testing in pilot scale, together with the five GAC products. The screening of PAC products in lab scale showed different, but typical dose response curves, Figure 19.

![Figure 19: Dose response curves from PAC screening in bench-scale with 60 minutes contact time.](image)

To achieve 95% removal of the 22 APIs, the bench scale tests indicated that the best performing PAC products must be applied with a dose of 30 mg/L, which consequently was set as the initial dose for the pilot tests. Figure 19 shows the importance of selecting a proper PAC product for treatment of a specific wastewater. PAC A, B and C in the figure legend above were the selected products, later used in the pilot test.

4.5.2 Experiences from the pilot plant operation of GAC and PAC

A comparison of the pilot plant performance, with the design values, showed that the hydraulic capacity of the GAC filters was influenced by the effluent quality, mainly regarding particles, measured as suspended solids (SS). During the first tests, all performed at Käppala WWTP, the SS concentration was below 1 mg/L in the regular effluent and the design hydraulic capacity in the GAC filters was maintained. This was also the case in filters with small GAC particle sizes (MESH 12*40), and with the applied backwashing, for removal of accumulated particles, two times a week. Käppala WWTP has full-
scale sand filters as a final treatment step and these lowered the SS concentrations below 1 mg/L. After relocation from Käppala to Uppsala WWTP, the GAC filters clogged after only one day of operation. The backwash frequency was therefore increased to five days a week. The immediate hypothesis was that presence of suspended solids in the effluent, partly in form of flocs from the final chemical treatment for removal of phosphorous, clogged the GAC filters. Installation of shallow sand filters, upstream the GAC-lines, partly reduced the clogging, but the hydraulic capacity of the GAC filters was still 50-80% of the capacity achieved at Käppala. The limited hydraulic capacity of the GAC filters in the pilot plant remained at Västerås WWTP. By shifting to a coarser GAC product (MESH 8*30), the hydraulic capacity of the GAC filters was restored to 90%, but unfortunately the removal efficiency of APIs was reduced to 75-85%, in stead of the typically 95%. From this study, it is unclear if it is the coarse GAC grains themselves, or just the selected product, that gave the lower removal efficiency. On contrary to the GAC lines, PAC lines were not influenced by the particles in the regular effluent. The final sand filter in each PAC line was backwashed once a week and maintained a high hydraulic capacity at all WWTPs visited.

The risk of clogging of the GAC filters was an important finding during the pilot tests and must be taken into account before GAC filters are implemented in full-scale. Typical design values for full-scale filters are hydraulic loads of 6-10 m/h\(^2\)\(^9\) (the pilot filters had a design value of 5.7 m/h). Measures to avoid clogging can be an installation of preceding sand filters, or to find GAC products that have both coarse grain fractions, and high adsorption capacity.

The PAC lines were in continuous operation and worked well during the tests, after solving problems with the dosing of PAC-slurry. Initially, clogging of the narrow hoses (2 mm inner diameter) for PAC slurry dosing, was solved by installing a sieve on the hose inlet, to prevent coarse grains of PAC to block hose couplings. The PAC lines were operated without limitations in the hydraulic capacity, throughout the basin trains. Minor difficulties to control the feed pumps occurred, due to wear and tear of the helical rotor pumps.

Backwashing of the sand filter was performed once a week, but long-term tests showed that even after 14 days without backwashing, the capacity of the sand filter was sufficient for the applied feed flow, corresponding to linear filter velocity of 2-7 m/h in the sand filters. The applied linear filter velocity was lower than the value discussed in recent publications\(^2\(^9\)\(^5\), but was chosen from experiences from long-term operation of full-scale sand filters in regular Swedish WWTPs\(^2\(^9\)\(^6\),\(^2\(^9\)\(^7\).
4.5.3 Adsorption of pharmaceutical residues - GAC

Two GAC lines were operated in the pilot plant to evaluate two GAC products in parallel. In total five GAC products were tested during the pilot test. GAC A–E correspond to product/supplier; GAC A: Aquacarb 207C/Chemviron; GAC B: Aquasorb 5000/Jacobi; GAC C: Filtrasorb 400/Chemviron; GAC D: GPP-20/Chemviron; GAC E: Carbsorb 30/Chemviron. GAC A and D were used in Käppala, GAC B was used in Käppala and Uppsala, and GAC C and E were used in Uppsala and Västerås.

Treatment of wastewater with GAC filters is a long-term process, with continuous adsorption and desorption of organic substances onto the internal surfaces of the activated carbon, trying to reach equilibrium after changes in wastewater composition.

The adsorption in the GAC filters was followed by breakthrough curves for each API, where the ratio of concentration in the advanced treated and the regularly treated wastewater, \((C/C_0)\), was plotted versus the number of passed bed volumes (BV). The patterns of the breakthrough curves differed for the APIs but also among GAC products. To demonstrate this, breakthrough curves for three indicator APIs, stated in EU and Switzerland, plus a fourth API, irbesartan were compared, Figure 20.

The comparison showed that GAC A and E had the lowest removal capacity, i.e. breakthrough occurred earlier, at a lower number of treated BV, than for the other products, GAC B, C and D. These products had more than five times as high capacity to adsorb clarithromycin and irbesartan than GAC A and E. The ratio \(C/C_0\) scattered mainly due to variations in the API concentration in the WWTP effluent. GAC E was selected and used to improve the hydraulic capacity of the GAC-filters, as described above, and unfortunately of the expenses of adsorption capacity.

Breakthrough curves for all 22 substances were plotted to determine the specific adsorption capacity of the GAC products. Generally, GAC A and GAC E showed lower specific adsorption capacities, compared with the other products, except for high adsorption capacities for carbamazepine and trimethoprim.
Figure 20: Breakthrough curves from the GAC pilot experiments. Breakthrough, $C/C_0$, the ratio of concentration in advanced treated wastewater, $C$ and the concentration in the regularly treated wastewater $C_0$, is plotted against treated bed volumes (BV) and displayed for carbamazepine, clarithromycin, diclofenac and irbesartan. Dashed lines between the discrete samples are added to improve visual interpretation.

The different GAC products adsorbed individual API very differently, even though most APIs contain one or more aromatic groups, which lead to the conclusion that not only aromaticity of the molecules contributes to adsorption onto activated carbon. For the individual APIs, the specific adsorption capacities, varied between 0.03 μg fexofenadine per g dry GAC, to 23 μg metoprolol per g dry GAC, at a breakthrough level ($C/C_0$) of 0.05. The adsorbed specific amount of APIs was approximately linear versus the number of treated bed volumes, Figure 21, which indicates that the for the sum of the selected 22 APIs has not reach its maximum value. The values of specific adsorption capacities achieved were much lower than previously reported values in distilled water and surface waters\textsuperscript{[98]}. This indicates the matrix effect, when wastewater bulk organic materials compete with APIs in the adsorption onto the available adsorptive surfaces of the activated carbon. This is a reasonable hypothesis, looking at the average removal of total organic carbon (TOC) from the regular effluent, which was 43% in the GAC-lines.
The average carbon usage rate (CUR) of GAC, to remove 95% of the selected APIs, was 110 g/m$^3$ wastewater, for all five GAC products and at all three WWTPs studied. The corresponding carbon usage rate for the three, best adsorbing GACs were: GAC B<43 g/m$^3$ (at 6440 BV), GAC C<41 g/m$^3$ (at 10210 BV) and GAC D<28 g/m$^3$ (at 16130 BV). Final values could not be determined, since the test periods were too short to reach 5% breakthrough, corresponding to 95% removal efficiencies of the selected APIs. The remaining two products A and E showed much higher carbon usage rates: GAC A 230 g/m$^3$ (at 2290 BV) and GAC E 170 g/m$^3$ (at 2360 BV). No general explanation for the differences in carbon usage rates and adsorption capacity was found from the available GAC product characteristics. However, the author observed that a higher methylene blue number (MBN) corresponded well to a higher
adsorption of APIs, i.e. products GAC A and E both have an MBN of 230 and showed lower removal than GAC B and C with an MBN of 260. Consequently, MBN should be further evaluated, as a guiding parameter in the selection process of GAC products for removal of APIs.

4.5.4 Adsorption of pharmaceutical residues - PAC

Three different PAC products were used in this work. PAC A—C correspond to product/supplier; PAC A: Pulsorb C/Chemviron; PAC B: Aquasorb MP20/Jacobi; PAC C: Aquasorb 5000P/Jacobi. PAC A was used in the tests at Käppala WWTP and Uppsala WWTP, PAC B was used in tests at all three plants and PAC C was used in tests at Uppsala WWTP and Västerås WWTP. The applied fresh PAC dose was initially set to 30 g/m$^3$, based on lab tests, and then stepwise lowered during the treatment campaigns. The dose of 30 g/m$^3$ was more than sufficient to reach 95% overall removal, in both Käppala and Uppsala, while 94% overall removal was achieved in Västerås, by applying a reduced fresh dose of 15 g/m$^3$. The accumulation of PAC product in the treatment tanks due to recirculation, contributed to an increase of the removal efficiencies of APIs. The average specific adsorption capacity at a breakthrough of 0.05, corresponding to 95% removal efficiency for the three PAC products, varied between 1.0 µg/g (clarithromycin) and 23 µg/g (metoprolol), the latter had the same high value as for the best GAC product.

4.5.5 Removal efficiencies of individual pharmaceutical residues

The removal efficiencies of individual APIs were related to the carbon usage rate (CUR) for both GAC and PAC. CUR for GAC was calculated relating the loaded amount of GAC to the treated volume of wastewater. In the PAC systems, the accumulated amount of PAC was related to the treated volume of wastewater, Figure 23.

Removal efficiencies of individual for GAC products

In order to evaluate the removal efficiency for the individual substances related to CUR, results from all discrete calculations of removal efficiencies were divided into dose intervals. The individual removal efficiencies of APIs showed that 8 out of 22 substances were removed more than 95% at a carbon usage rate of 30-100 mg/L i.e. 30-100 g/m$^3$, for all experiments and GAC products, Figure 22.
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Figure 22: Average removal efficiencies of APIs for the five GAC products in the pilot experiments divided into carbon usage rate intervals.

The indicator APIs, carbamazepine and clarithromycin were removed more than 95% and diclofenac to 93% in this carbon usage rate interval. A carbon usage rate, which exceeded 100 mg/L, did not result in a considerable improvement of removal, mainly due to poor removal efficiencies for GAC A and E. In the present test, the removal efficiencies were in the same range or higher than in other reported evaluations of GAC performance in regular effluent wastewater\(^{10,299,300}\). Comparisons of removal efficiencies can be difficult to make, since the number of treated bed volume, at the sample occasions, at pilot or full-scale plants, are sometimes missing in published papers.

Removal efficiencies of individual for PAC products

Analogous to the evaluation of removal efficiencies in the GAC pilot tests, all the PAC experiments were sorted in PAC dose intervals. The individual removal of APIs showed that 16 out of 22 substances were removed more than 95% by a carbon usage rate of <30-100 mg/L for all experiments and the three PAC products. Concerning the indicator APIs, carbamazepine, clarithromycin and diclofenac, all were removed more than 95% in the carbon usage rate interval 30-100 mg/L, Figure 23.
Seven of the 22 APIs were removed less than 95% at all CURs, although fexofenadine, bupropion and venlafaxine were removed by 94%, close to the target value. Fluconazole, mirtazapine, diltiazem and memantine were clearly removed less than 95%, but PAC removed these substances better than GAC did, by approximately 10 %-units. However, the added PAC dose in most experiments with was 26—30 mg/L, which was in accordance with the lab test, performed to select proper PAC products and doses. By the last pilot tests, which were performed in Västerås, the dose was lowered from 26 mg/L to 15 mg/L. By this decrease in PAC dose, the average removal efficiency dropped from 99% to 95%. The removal efficiencies were higher than, or similar to, previously reported results from PAC applications.\textsuperscript{301,302}

### 4.5.6 Mechanisms for adsorption of APIs on GAC and PAC

In general, the specific adsorption of APIs was higher on PAC than GAC, most probably due to the smaller particle size of PAC, which makes the pores more accessibly for diffusion and adsorption of APIs. The comparison of GAC and PAC in this work was based on all products tested. Still, if the two low-performing GAC products were excluded, PAC showed a higher specific adsorption, although less pronounced.

A ranking of the removability of the 22 APIs, where the results from GAC and PAC tests were combined, Table 14, showed that the first nine APIs, i.e. with the highest removability, are all positively charged at normal wastewater pH. This indicates that the net surface charge of the activated carbon was negative when it was in operation, likely as a result of co-adsorption of organic matter.
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present in the effluent wastewater. The higher removability of positively charged APIs by activated carbon has previously been reported\textsuperscript{176,302}.

Table 1. Ranking of removability, equally based on removal efficiency and % below LOQ. Molecular structures are show in declining removability, ordered left-to-right then top-to-bottom.

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>GAC</th>
<th>PAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisoprolol</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Atenolol</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Sotalol</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Citalopram</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Codeine</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Tramadol</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Bupropion</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Flecaïnide</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>17</td>
<td>16</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>Memantine</td>
<td>18</td>
<td>22</td>
</tr>
</tbody>
</table>

The four best ranked substances, bisoprolol, atenolol, sotalol and trimethoprim, were all positively charged at the actual wastewater pH 6.7–7.7 and they all contain at least one aromatic group and hydrogen-bond donor/acceptor groups, Table 14.

The four poorest ranked substances were memantine, fluconazole, irbesartan and clindamycin. Memantine is positively charged at actual wastewater pH 6.7–7.7, has a low molecular weight and a compact molecular structure and is not aromatic, the latter has proven to be a disadvantage for adsorption onto activated carbon\textsuperscript{303}. Fluconazole is neutrally charged at wastewater pH and is therefore more dependent on hydrophobic interactions with the activated carbon, than positively charged substances. The low log D value of fluconazole indicates predominantly hydrophilic properties, which are unfavorable for the adsorption. Irbesartan is negatively charged, which contributes to a low
adsorption. Clindamycin is positively charged at wastewater pH, is not aromatic and it has a moderate to low log D value, that can explain the low adsorption.

In summary, activated carbon, in form of treatment lines with GAC and PAC, was shown to be an efficient technology for the removal of APIs in the effluents of municipal wastewater treatment plants. In the designed pilot treatment lines, 95% removal efficiency was reached for almost all tested substances. Large variations in specific carbon usage rates were observed between GAC products, but also between different APIs.

The APIs to be regulated in effluent standards will influence the selection of GAC and PAC products to a specific WWTP and a variation of both GAC and PAC qualities should be screened before acquisition, to find products with lower carbon usage rate and thereby lower cost. Concerning GAC, screening with respect to hydraulic capacity is also important before purchase.

PAC seemed to have a generally higher removal efficiency at a lower carbon usage rate than GAC, but well-performing GAC products approached the performance of PAC, as indicated by the results.

4.6 Examination of process parameters in the PAC lines (Paper IV)

The PAC lines were designed based on literature data and bench-scale tests of the dependency of contact time and removal efficiency. The comparable testing in pilot scale described above showed that a fresh PAC dose of 15-16 mg/L, a contact time of 60 minutes and recirculation of used PAC to the first contact tank was sufficient to remove 95% of the 22 APIs in the study. In the pilot tests, the PAC dose was successively lowered from 30 mg/L and finally levelled out at 15 mg/L to maintain 95% removal. The research question below was raised to examine the dependency of contact time and recirculation on the removal efficiency of APIs in a PAC system.

4.6.1 Contact time in contact tanks for wastewater and PAC in pilot scale

Three contact times in the contact tanks were evaluated: 30, 60 and 120 minutes, at a fresh PAC dose of 15 mg/L and under recirculation of used PAC. The corresponding contact time in one contact tank was 10, 20 and 30 minutes respectively. The overall removal efficiencies of the APIs were marginally
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Improved by a longer total contact time than 30 minutes. The removal efficiency increased from 95% at 30 minutes, to 97% at 60 minutes and 98% at 120 minutes. A long contact time was however beneficial for the removal efficiency of clindamycin, fluconazole, irbesartan, oxazepam and tramadol. They all approved the 95% removal level, when increasing the contact time from 60 to 120 minutes. Memantine and venlafaxine did not reach more than 80% removal efficiency, even at the longest contact time.

Similar test with 60- and 120-minute contact times were performed, but without recirculation of used PAC. The use of recirculation increased the overall removal efficiency of APIs from 92 to 97%, at 60 minutes contact time, and from 96 to 98%, at 120 minutes contact time. A comparison of removal efficiencies for individual APIs in systems, with and without recirculation of used PAC, showed that recirculation was beneficial for the removal of carbamazepine, fluconazole, Irbesartan, sotalol, tramadol and trimethoprim, Figure 24. Experiences from other studies showed that 50-67% lower doses of PAC can be applied through recirculation of PAC from different separation units, following the contact tanks\textsuperscript{301,304}.  

\textbf{Figure 24:} Effects on the removal comparing operation with recirculation to the first contact tank (R1) and without (RX) at nominal contact times of 60 min (top) and 120 min (bottom). Values below 95% removal and below the LOQ are marked with an asterisk (*). Error bars indicate the standard deviation of duplicate experiments.
Recirculation had a relative larger positive effect of removal efficiency of APIs at the shorter contact time of 60 minutes. In summary, the aim of 95% overall removal of the APIs was reach by a fresh dose of 15 mg PAC/L, at a contact time of 30 minutes and with recirculation of used PAC. However, the removal efficiency of some less readily adsorbed APIs, increased with longer contact times and applied recirculation of used PAC. Again, the selected set of APIs in effluent standards will influence the design of the PAC process at a specific WWTP.

4.6.2 Recirculation release point of used PAC

The influence on removal efficiency of different recirculation release points, i.e. discharge of the recirculation stream to any of the three contact tanks (configuration R1-R3), was evaluated at 30 min contact time and at fresh PAC dose of 15 mg/L. The removal efficiency was similar for all three recirculation points, with a slightly higher removal (95-96%) for recirculation to the first (R1) or second tank (R2) in series, compared to recirculation to the last tank (R3), which resulted in 94% overall removal efficiency, Figure 25.

Figure 25: Removal using the different recirculation configurations at 15 mg/L fresh PAC dose and 30 min nominal contact time. Values below 95% removal but decreasing the effluent concentrations below LOQ are marked with an asterisk (*). Diclofenac was below the LOQ in the WWTP effluent used in test R2. Error bars indicate the standard deviation of duplicate experiments.

Generally, a significant difference for the removal efficiency of individual APIs, due to recirculation point, could not be observed, but some APIs, clindamycin, irbesartan and tramadol, were mostly influenced by the position of the recirculation point, R1 was favorable over R2 and R3, Figure 25. Despite the expectations of a better performance in configuration R1, the removal in R2 was generally similar or slightly better for five out of nine APIs, while three of nine APIs performed better in R1. In summary, comparison of different
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Discharge points for the recirculation showed that recirculation to R2 resulted in the best performance with respect to removal efficiencies of individual substances.

With regards to 95% overall removal efficiency, the classical setup, i.e. recirculation of used PAC to R1 and R2, must be used. Regarding the indicator APIs, the only example of insufficient removal was for carbamazepine in configuration R3, where the removal efficiency reached 92%.

4.6.3 Retention of PAC in a pilot plant – coagulation and flocculation

To achieve a high removal efficiency of APIs, the used PAC must be retained in the treatment unit. A discharge of PAC residues will carry adsorbed APIs to the next treatment step or to the receiving water. Additionally, an internal recirculation of PAC can be valuable to increase the retention of PAC, mainly through flocculation and sedimentation. Coagulants and flocculation aids have been applied in contacts tank to increase the retention of PAC\(^{176,305}\). Initial, separately performed pilot tests in this work showed that with an addition of both Al and Fe-based coagulants to the third contact tank, a faster blocking of the sand filter occurred. In connection with the evaluation of recirculation of PAC, a few experiments were performed with continuous addition of poly-aluminum chloride (PAX-XL350, Kemira) to the third contact tank, at 60 min contact time (configuration R1). Coagulant was added as 4 mg/L Al\(_2\)O\(_3\) and the fresh PAC dose was 12 mg/L. The overall removal efficiency of APIs was somewhat higher, 98% compared to 97%. The hydraulic capacity in the final sand filter was however significantly reduced. In opposition to the hypothesis, the PAC-sludge was less well retained in the treatment tanks, due to a bulkier sludge appearance. The major parts of the pilot tests were therefore run without coagulation and flocculation aids.

4.6.4 Treatment of secondary or tertiary effluent with PAC

Käppala WWTP has a sand filter step as a tertiary treatment to remove suspended solids and particle bound phosphorous. Uppsala WWTP and Västerås WWTP have not installed sand filters, which otherwise had produced tertiary effluent, so the latter WWTPs discharge different types of secondary effluent. Many WWTPs lack a tertiary treatment, so the pilot tests are valuable also from this perspective. During the pilot tests, it was obvious that the sand
Present investigation

filter removed particles, that otherwise had interfered with the GAC filters, while the PAC lines were not apparently affected.

During the evaluation of PAC process parameters, the average concentration of suspended solids in the secondary or tertiary effluent were 10 and 2 mg SS/L respectively. The hydraulic capacity in the PAC-lines was not influenced by the higher load of suspended substances, so the frequency of backwash in the final sand filter once every week was maintained.

In addition, the removal efficiencies of APIs seemed not to have been influenced by the higher concentrations of organic material in the secondary, than the tertiary effluent. Reconsider that the tests on tertiary effluent were performed with substantially higher fresh PAC doses, 25-37 mg/L, than the tests on secondary effluent, where the average dose was 15 mg/L, tests with lower PAC doses on tertiary effluents must be performed as well.

4.6.5 Influence of mixing with air on the pH in the contact tanks

During the experiments, a rapid increase in pH was observed in samples taken from the pumped effluent wastewater (pH 6.9), to the first (pH 7.3), second (pH 7.7) and third (pH 7.8) contact tank, which was hypothesized to be caused by stripping of carbon dioxide, which in turn is a result from the use of aeration as mixing aid. Bench-scale experiments showed a similar trend, when aeration was used for mixing, while the change in pH with mechanical stirring was negligible. pH influences the log D value differently for different APIs, which might influence the adsorption. No considerable difference in adsorption is however expected, due to the probability that PAC surfaces have a predominantly negative charge at pH 7, mainly because of co-adsorption of organic matter, present in the wastewater.

4.6.6 Conclusions from the evaluation of PAC parameters

The PAC-based systems, with recirculation, in a series of three tanks, achieved high removal of APIs, both from secondary and tertiary effluents. Recirculation to improve the removal of APIs or lower the fresh PAC dose to maintain a proper removal efficiency, should be either established to the first or the second tank. Physical retention of PAC, e.g. by use of sand filtration is required to maintain a high recirculation degree of PAC, but also to prevent PAC with adsorbed APIs to reach the effluent, which will lower the removal efficiency. Depending on the effluent standards and PAC product selected, the
contact time and PAC dose must be varied to achieve different degrees of removal efficiency, both with respect to the overall removal of pharmaceuticals, and with respect to the removal of individual APIs.

4.7 Treatment with ozone (Paper V)

Ozonation was the third technology to be evaluated for removal pharmaceutical residues from wastewater, in addition to nanofiltration and activated carbon. The aim for the tested removal technologies was to achieve 95% removal of a selected set of 22 APIs, initially detected as frequently occurring in Swedish municipal wastewater.

During the field tests, two ozonation units were operated in parallel in the mobile pilot plant when it was in operation at Käppala WWTP, Uppsala WWTP and Västerås WWTPs. The ozonation units were operated also in parallel with the GAC and PAC units (described in paper III-IV), to facilitated comparison of different treatment technologies for removal of pharmaceutical residues.

4.7.1 Removal efficiencies of pharmaceutical residues by ozonation

Paper V describes the results from a relative short period, two weeks out of 57 weeks of the pilot tests, when ozonation for removal of APIs was evaluated at Käppala and Uppsala WWTPs, in connection with ecotoxicity tests. The first week, at Käppala WWTP, is more representative for the ozonation tests during the remaining 55 unreported weeks, than the presented second week, at Uppsala WWTP, when problems with one feed pump, disturbed the ozone dosing and prolonged the hydraulic retention time.

During the described periods in paper 5, ozonation line 1 was operated with the 5 m high contact column 1 (CC1), which had an operational volume of 83 L, where the reaction between ozone and organic substances occurred during on average 36 and 47 minutes (Käppala WWTP and Uppsala WWTP, respectively). The longer hydraulic retention time in Uppsala was an effect of lost capacity of the feed pump. The ozonated wastewater passed the aerated stripping tank for the removal of potential residues of free ozone, that otherwise might interfere with the biotests. During the tests at Käppala WWTP, parts of the ozonated wastewater also passed a sand filter with a surface load of 2.7 m/h, for a potential removal of biodegradable products,
released in the ozonation during the tests. In Uppsala the same pilot sand filter was used to study the effects of biomarkers in rainbow trout, only adding a sand filter to treat the regular effluent wastewater.

During the tests, the ozone doses were 7 g/m$^3$ (specific ozone dose 0.92 g O$_3$ per g TOC) in Käppala WWTP and 5.4 g/m$^3$ (specific ozone dose 0.82 g O$_3$ per g TOC) in Uppsala WWTP. The average removal efficiencies of the 22 APIs were 89% and 87% in Käppala WWTP and Uppsala WWTP, respectively. These results were mainly in accordance with the results from a published lab study on effluents from six Swedish WWTPs, spiked with APIs$^{306}$. The average removal of 13 APIs present in regular effluent from three of the six WWTPs in the lab study were 81% and 71%, at the corresponding ozone doses of 7.0 and 5.4 g/m$^3$, respectively. The corresponding removal efficiencies in Käppala and Uppsala were 81% and 79%, respectively. The three selected WWTPs had similar average DOC concentrations in their effluents, 9.1 g/m$^3$ in both lab and pilot groups of the WWTPs. A direct comparison of the 13 selected APIs, shows that the removal efficiencies of the individual APIs were scattered between the two groups of WWTPs, Figure 26.

**Figure 26:** Correlation of removal efficiencies in lab scale test (literature data) and performed pilot tests at Käppala and Uppsala WWTPs. The ozone doses are marked with orange for 5.4 g/m$^3$ and blue for 7 g/m$^3$ respectively.
The results indicate that the removal efficiency was higher in pilot scale for many APIs, than in lab scale at an ozone dose of 5.4 g/m$^3$, but similar results were achieved in both scales at an ozone dose of 7 g/m$^3$. One reason for this disagreement might be the relatively longer retention time in the contact tank at 5.4 g/m$^3$ in the pilot tests in Uppsala.

At Käppala WWTP, the ozonation resulted in 89% overall removal efficiency of APIs by the addition of 7 g/m$^3$, while the number of quantified APIs was reduced from 24 to 3 by the ozonation. At Uppsala WWTP, the ozonation of effluent wastewater resulted 87% overall removal efficiency of APIs by the addition of 5.4 g/m$^3$ and the number of quantified APIs was reduced from 25 to 10 by the ozonation. It seems that the level of ozone dose is more important, than the retention time in the contact tank, to remove as many APIs as possible. The removal efficiencies of individual APIs were not uniform at a specific ozone dose, but differed from the average, Figure 27.

![Figure 27](image-url) - Removal efficiencies of the APIs quantified in effluent in Käppala WWTP and Uppsala WWTP during the pilot test during the evaluation with biomarkers.

Seven of the 26 APIs quantified were removed to more than 95%. Half the number of the 26 APIs had a removal efficiency between 80-95%. In the interval 50-80% removal efficiency, five APIs were observed, among them flecanide and fluconazole, which are reported to be only moderately removed by ozonation\textsuperscript{307}. 
Mechanisms for removal of APIs by ozonation

The reason for the relatively low removal of flecanide and fluconazole can be that the molecules have electron-withdrawing fluoro groups, that also shield from ozone attack. Bupropion was the only API removed less than 50%. The poor removal of bupropion is not apparent, simply by looking on the chemical structure, since bupropion contains an aromatic functional group and should be vulnerable for an attack of ozone. The limited removal seems to be a question of ozone dose in relation to DOC concentration, which in the two pilot tests was 0.68 g ozone per g DOC on average, but more likely one disregarded shielding effect or simply deconjugation of some of the in human formed metabolites, back to the parent substance. In the controlled lab study on real wastewater effluent, but spiked with APIs to individual concentrations of 1 µg/L, a similar specific ozone dose of 0.74 g per g DOC resulted in 45% removal of bupropion, which is moderately higher than the achieved results in pilot scale\textsuperscript{306}. The low ng/L concentration of bupropion in un-spiked effluent might also have influenced the lower removal efficiency, due to lower reaction rates at lower concentrations according to first order kinetics.

4.7.2 Selection of ozone dose and hydraulic retention time in contact column

Dose-response tests were done in the pilot plant during the tests at Käppala WWTP to find a proper ozone dose to remove 95% of the APIs. By previous in-house ozonation tests it was shown that at high ozone doses, e.g. 15 g/m\textsuperscript{3}, caused adverse effects in test organisms, so as low dose of ozone as possible to remove 95% of the APIs was favorable\textsuperscript{109}, but also beneficial from the perspective of resource consumption. Based on these reflections, an ozone dose of 7 g/m\textsuperscript{3} (0.77 g per g DOC) was selected as target value for the long-term operation in Käppala WWTP and Uppsala WWTP, although the average removal of APIs was 90% at 7 g/m\textsuperscript{3} in the dose-responds tests and thus below the goals of 95% removal efficiency.

As a comparison, the lab tests described in connection with Figure 26, demanded 0.93 g O\textsubscript{3} per g DOC to achieve 90% removal efficiency of 13 overlapping APIs. The applied specific ozone dose 0.77 g O\textsubscript{3} per g DOC, during the pilot test, resulted in 80% removal efficiency of 13 overlapping APIs.

The ration between these doses and corresponding removal efficiencies confirms earlier observations that the relation of ozone dose to removal
efficiency is not linear, but logarithmic, more apparent at higher removal efficiencies, >80%.

During the dose-response tests, the hydraulic retention time (HRT) in the contact column was 36 minutes, equal to the total HRT in one GAC line. The applied HRT in the ozonation contact column was based mainly on preceding tests in the mobile pilot plant, where the necessary HRT showed to depend on API concentrations i.e. a greater sum of concentrations of APIs in the effluent demanded a longer HRT (unpublished data). Strictly from this evaluation, the HRT could have been set to 30 minutes, but to add a margin of safety for occasional high concentrations of APIs during the biotests, 36 minutes was chosen as HRT in the ozonation contact tank.

By ozonation, the pilot tests showed that the kinetics of degradation of individual APIs varied substantially. Again, the selection of targeted substances, and their concentrations, will influence the applied treatment, here in form of the length of the HRT in the ozonation contact tanks (unpublished data). During the full period of pilot tests, the dependence of pH, temperature and wastewater pressure on removal efficiency, were tested and evaluated in addition to the effect of ozone dose and HRT (data not shown).

In summary, the aim of 95% overall removal of APIs was not consistently reached by ozonation, but with an appropriate ozone dose of 5-7 g/m³, ozonation reach 85-90% overall removal efficiency of the selected APIs. The ozone dose must be adopted in relation to the concentration of bulk organic substances in the wastewater, to amount at least a ratio of 0.77 g O₃ per g DOC, to reach 89% removal efficiency of 24 selected APIs. The HRT in the ozonation contact tanks had to be 30 minutes for the concentration level of APIs during the biotests. The sand filter, following the ozonation, did not improve the removal of pharmaceuticals, but the TOC concentrations decreased by 5%. Fluconazole and bupropion were the most resistant APIs to ozonation.
**Area 4)** Does the present discharge of APIs lead to biological consequences in the aquatic environment?

### 4.8 Evaluation of treatment technologies by biomarkers (Paper V)

To validate the removal efficiency of advanced treatment technologies, chemical analysis of pharmaceutical concentrations is crucial. It is however a risk that substances, not being analyzed or potentially formulated as byproducts in ozonation, will be harmful in the aquatic environment. Use of biomarkers, complementary to the chemical analysis, offers a good indication of the presence or production of harmful substances in untreated or treated wastewater.

Biomarkers reflect interaction between a biological system and a potential chemical, physical or biological hazard and were used in parallel with chemical analysis, to evaluate the effluents from ozonation and activated carbon. Biomarkers used in the evaluation of polluted water are often linked to the metabolism of hazardous substances.

The set of biomarkers applied in this study, integrates responses both of known and unknown chemical substances. The biomarkers were measure both on mRNA and protein level and were chosen to span the effects of a large variety of possible substances, i.e. aryl hydrocarbons estrogens (EROD, CYP’s), metals (MT), oxidative stress inducers (HSP 70, SOD) and pregnane X receptor agonists (CYP3A45). Rainbow trout (Oncorhynchus mykiss) was used as biological system and samples were taken from both gills and liver to allow measurement from both a more rapid and slower reacting system.

In total three exposures tests on rainbow trout were performed, one on lab scale treatment in Käppala WWTP, one on pilot scale treatment in Käppala WWTP and one on pilot scale treatment in Uppsala WWTP. The initial exposure test in Käppala was performed on effluents from lab scale setups of ozonation and activated carbon, which was constructed to supply supporting data for the design of the treatment lines in pilot scale. The initial exposure test was also used to select relevant biomarkers for the subsequent exposure tests in Käppala and Uppsala.

In the lab scale ozonation, the added ozone dose was 7 g O₃/m³ and it resulted in 65% average removal efficiency of APIs, which indicated a limited transfer of ozone to the wastewater, likely due to the low column (0.9 m). Later performed pilot scale tests, with addition of 7 g O₃/m³ in a 5 m high column,
Results and discussion

Gave 87-90% removal efficiency, showing the shortcomings of a low contact column. The activated carbon filtration (GAC) resulted in 99.8% removal of APIs.

The relatively poor removal of APIs in the lab scale ozonation plant was also reflected in some biomarker responses. The liver induction of CYP1A1 and CYP1A3 transcription in fish exposed to ozonated water was probably due to remaining persistent aryl hydrocarbon receptor agonists. This has been shown earlier, when the ozone dose was insufficient to remove the target substances.

In pilot scale, the first exposure of rainbow trout to untreated and treated effluent was performed at Käppala WWTP for one week, when ozonated, ozonated plus sand filtered and granular activated carbon treated effluents, were compared with the negative control; tap water and the positive control: effluent from the wastewater treatment plant.

Chemical analysis showed that both activated carbon filtration and ozonation, with the addition of 7 g/m³, resulted in 89% average removal of APIs in the effluent wastewater. These treatments significantly reduced EROD activity to levels below that recorded in rainbow trout held in regular effluent and even below that in fish held in tap water.

The induction of CYP1A1 and CYP1A3 transcription in the liver in fish exposed to effluent from the lab scale ozonation described above, was significantly reduced in the pilot scale ozonation, likely due to a taller contact column, which facilitated a higher removal of AhR agonists. Furthermore, the indicated increase in transcription of heat shock protein 70 (HSP70) and metallothionein (MT) in gills of fish, exposed to the ozonated effluent in the lab study, could not be confirmed in the pilot scale, indicating that the ozonation did not induce oxidative stress in the studied biological system.

The second major exposure on rainbow trout of effluent from pilot scale treatment lines with sand filter, ozonation and granular activated carbon was performed for one week at Uppsala WWTP. Tap water was used as negative control and regular effluent was used as positive control. During the exposure, the ozonation, with the addition of 5.4 g/m³, resulted in 87% removal efficiency and activated carbon filtration removed 95% of the APIs.

Fish exposed to regular effluent, with or without sand filter, showed significantly induced Gill EROD activity and increased the transcription of cytochrome P450 (CYP1As and CYP1C3) in liver, compared to activity and transcription in fish held in tap water. This has also been observed in other
EROD activity in fish exposed to GAC-treated effluent was lower and EROD activity in fish exposed to ozonated water was slightly, but significantly higher than in those exposed to tap water.

Ozonation means addition of very oxidative ozone into the wastewater, which led to the application of two relevant biomarkers, the heat shock protein 70 (HSP70) and the superoxide dismutase (SOD), which response to oxidative stress in fish. The tests did not show any increased oxidative stress response in the fish, due to ozonation in pilot scale, on contrary to the results in lab scale, where the ozone transfer was limited. Previous studies have shown increases in HSP70 responses in ozonated effluent, at ozone doses of 5 and 15 g/m³, but it was not observed in the pilot tests in Käppala and Uppsala. The ozonated effluents were however stripped by aeration to remove free ozone, thus no effect could be expected from the O₃ molecule itself. The conclusion from the ozonation tests were that xenobiotic byproducts and/or oxygen radicals were not present in concentration high enough to evoke response from the chosen biomarker in gill and liver.

In summary, biomarker responses indicated that the effluents from WWTPs, using regular treatment, contained substances to a concentration that elevated some of the biomarkers (EROD, CYP1’s), specifically responding to aryl hydrocarbon receptor (AhR) agonists. However, these responses disappeared by the applied advanced treatment technologies. GAC treatment and ozonation significantly decreased the concentrations of APIs present in regular effluent water. Furthermore, ozonation did not increase the oxidative stress responses in the fish. Consequently, GAC treatment and ozonation are considered to be suitable and implementable treatment technologies to remove biologically active contaminants in wastewater. To increase the validity of this conclusion, repeated and additional tests with biomarker responses, especially concerning chronic toxicity, are recommended.

### 4.9 Comparison of treatment technologies

The tested treatment technologies have shown good overall removal efficiencies for APIs, but for nearly all technologies, limitations in the removal efficiencies of some individual APIs were observed. A comparison of the removal efficiency for the total set of APIs, shows that the overall removal efficiency was higher than 80% for all tested technologies, Table 15.
Results and discussion

Table 15. Ranking of APIs in descending order of average removal efficiency (RE). O3=Ozonation; GAC=Granular Activated Carbon; PAC=Powdered Activated Carbon and NF=Nanofiltration.

<table>
<thead>
<tr>
<th>Pharmaceutical</th>
<th>O3 RE [%]</th>
<th>GAC RE [%]</th>
<th>PAC RE [%]</th>
<th>NF RE [%]</th>
<th>Average RE [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfuzosin</td>
<td>92</td>
<td>-</td>
<td>99.6</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>97</td>
<td>89</td>
<td>99.9</td>
<td>90</td>
<td>94</td>
</tr>
<tr>
<td>Clarithromycine</td>
<td>92</td>
<td>90</td>
<td>99</td>
<td>-</td>
<td>94</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>98</td>
<td>85</td>
<td>98</td>
<td>-</td>
<td>94</td>
</tr>
<tr>
<td>Atenolol</td>
<td>87</td>
<td>94</td>
<td>98</td>
<td>95</td>
<td>94</td>
</tr>
<tr>
<td>Tramadol</td>
<td>98</td>
<td>88</td>
<td>95</td>
<td>92</td>
<td>93</td>
</tr>
<tr>
<td>Codeine</td>
<td>90</td>
<td>84</td>
<td>99.6</td>
<td>99</td>
<td>93</td>
</tr>
<tr>
<td>Sotalol</td>
<td>91</td>
<td>88</td>
<td>99</td>
<td>-</td>
<td>93</td>
</tr>
<tr>
<td>Citalopram</td>
<td>97</td>
<td>82</td>
<td>94</td>
<td>96</td>
<td>92</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>-</td>
<td>84</td>
<td>99</td>
<td>-</td>
<td>92</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>96</td>
<td>79</td>
<td>99</td>
<td>-</td>
<td>91</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>91</td>
<td>92</td>
<td>99.7</td>
<td>77</td>
<td>90</td>
</tr>
<tr>
<td>Bisoprolol</td>
<td>86</td>
<td>82</td>
<td>99.7</td>
<td>-</td>
<td>89</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>90</td>
<td>82</td>
<td>95</td>
<td>-</td>
<td>89</td>
</tr>
<tr>
<td>Irbesartan</td>
<td>83</td>
<td>86</td>
<td>97</td>
<td>-</td>
<td>88</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>96</td>
<td>72</td>
<td>98</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Flecaínide</td>
<td>77</td>
<td>88</td>
<td>99.5</td>
<td>-</td>
<td>88</td>
</tr>
<tr>
<td>Mirtazapine</td>
<td>82</td>
<td>81</td>
<td>98</td>
<td>92</td>
<td>88</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>86</td>
<td>-</td>
<td>-</td>
<td>89</td>
<td>88</td>
</tr>
<tr>
<td>Oxazepam</td>
<td>71</td>
<td>85</td>
<td>99</td>
<td>87</td>
<td>85</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>80</td>
<td>72</td>
<td>93</td>
<td>-</td>
<td>82</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>58</td>
<td>74</td>
<td>91</td>
<td>-</td>
<td>74</td>
</tr>
<tr>
<td>Memantine</td>
<td>68</td>
<td>66</td>
<td>88</td>
<td>-</td>
<td>74</td>
</tr>
<tr>
<td>Bupropion</td>
<td>28</td>
<td>50</td>
<td>98</td>
<td>-</td>
<td>58</td>
</tr>
</tbody>
</table>

The results represent the outcome of typical operation of pilot plants and in the case of GAC, the average for the five tested GAC products is reported, including removal data for two low performing GAC products. The most striking result is the outstanding high removal efficiency, for most APIs, achieved by the PAC lines. The substances in Table 15 are sorted by average
removal efficiencies, in descending order, with the purpose to show the easiest removable substance at the first row.

In the list above, the APIs reported to have adverse effects on aquatic organisms: diclofenac, oxazepam and the antibiotics (trimethoprim, clarithromycine and clindamycine), were removed more than 80% by any of the tested technologies, with the exception of oxazepam, which was removed only by 71% applying ozonation. However, the other technologies tested removed oxazepam in the range of 85%-99%. The LOQs of the applied analytical procedure were too high, to give values of the hormones reported to have adverse effects in aquatic organisms.

Three substances were removed less than 80% in average, due to relatively poor removal by ozonation and GAC. Fluconazole was, as discussed earlier, less effectively removed by ozonation, due to flouro groups in the molecule. The relatively poor average removal by GAC compared to PAC, comes from the two less well performing GAC products, of the five products tested, just comparing the three well-performing GACs, the same removal efficiency as PAC was reach, 91%, again showing the necessity to screen for an appropriate product before purchase. Memantine is, as described above positively charged at wastewater pH, has a low molecular weight and a compact molecular structure and is not aromatic, the latter has proven to be a disadvantage for adsorption on activated carbon, but also less reactive with ozone. Bupropion demands a higher ozone dose than 7 g/m³ to be removed – higher ozone doses can however be problematic due to the production of substances with adverse effects on water living organisms. The very high removal efficiency of bupropion by PAC was not observed for any of the GAC products, after treatment of 4000 bed volumes or more, which corresponds to a higher specific carbon usage rate of GAC, than PAC. Of the remaining APIs in treated effluent, fluconazole, used as an antifungal pharmaceutical, has the potential to inhibit the catalytic activity of CYP1A, but the concentrations of fluconazole in effluent after advanced treatment, seems to be so low, that inhibition will most likely not occur.

Investment and running costs for the studied treatment technologies are not explicitly evaluated in this work, but the author calculated these costs in two national and regional reports in 2008 and 2010. The total cost relationship was 1 : 1.7 : 5.3 for ozonation : GAC : nanofiltration and the cost constitutes an important part in the selection of treatment technology to be implemented in full scale.
In summary, all examined APIs, in this work could be removed to at least 88%. In most cases, PAC was the broadest working removal process to achieve very high removal efficiencies and PAC was the only process that fulfilled the study’s goal of 95% average removal of APIs. Aiming at more realistic removal efficiencies as 80%, all tested technologies are good candidates, but the total cost must be taken into account. For nanofiltration, the waste problem with retentate must be solved before nanofiltration can be implemented in the large scale.

**4.10 Spin-off of the ozonation pilot tests**

In late 2014, and not as a part in this thesis, the author designed Sweden’s first full-scale treatment for removal of micropollutants, including pharmaceutical residues, based on the mobile pilot tests within MistraPharma. Due to the limited budget and tense time schedule, ozonation was selected as treatment technology. The ozonation step has a capacity to treat wastewater from all 12 000 PE in the municipality of Knivsta, situated in the Stockholm Region. Knivsta WWTP was selected due to its reasonable size, 12 000 inhabitants, and the small receiving watercourse, that is heavily influenced by the treated wastewater.

After the construction phase, the ozonation in Knivsta WWTP constituted the final treatment step after the existing mechanical, biological and chemical wastewater treatment. By the full-scale operation, and thereby treatment of all effluent wastewater from Knivsta WWTP, the average removal efficiency, of the previously selected set of APIs (Table 15), was 80% at an ozone dose of 7 g/m³. This was in the same range as the results achieved in long term operation of the ozonation pilot plant, when comparing periods with the same concentration of organic material (TOC) in the effluent, typically 11 g/m³ (unpublished data). The Swedish Agency for Marine and Water Management, SwAM, funded a major part of the project in Knivsta.
4.11 Summary of work done and main results

In this work, the prevalence of pharmaceutical residues in municipal wastewater and in the aquatic environment has been studied, in parallel with the design, construction, operation and evaluation of the most promising treatment technologies in pilot scale, for removal of pharmaceuticals in municipal wastewater. The reason for carrying out this study comes from that pharmaceutical residues have been reported to have adverse effects in the environment and observations show that they are generally poor removed in municipal WWTPs.

Data collection showed that the specific consumption of pharmaceuticals varied between country regions and countries, but also altered with time. Differences in demography and prescription habits probably caused the variance in consumption of APIs between counties and the countries. One example from the Baltic Sea catchment area showed a large variation between the highest and lowest country specific consumption of carbamazepine in 2013, corresponding to a factor of 11. However, the availability and the details of the statistics on the consumption of pharmaceuticals varied a lot between countries, which complicated the modelling of the load of pharmaceuticals to the WWTPs in some countries and to the aquatic environment.

In the performed sampling campaign in the Baltic Sea, residues of pharmaceuticals were found in many locations, particularly outside major cities. The Baltic Sea is directly or indirectly receiving effluents from the majority of Swedish WWTPs, but also effluents from many WWTPs in the 13 other countries in the Baltic Sea catchment area. Carbamazepine was found all over the Baltic Sea, including in offshore locations, but the average concentration was low, 1.9 ng/L. In comparison to the estimated critical environmental concentration, carbamazepine itself, cannot be considered as a threat to the aquatic environment, not even in lakes with 100 times higher concentrations. However, due to the long turnover time and low removal rate of carbamazepine, a stock of over 55 metric tons of the substance has accumulated in the Baltic Sea.

In this thesis work, a developed grey box model predicted the analysed environmental concentrations of carbamazepine in the Baltic Sea sub basins and showed on a very long half-life of carbamazepine in the Baltic Sea, >3.5 years, which makes carbamazepine a good indicator of wastewater intrusion in natural waters. The grey box model displayed to be a simple and useful tool to predict environmental concentrations of organic substances in water, but the inaccessibility of open data of meteorological, hydrological and substance
consumption made the simulation more troublesome and potentially more inaccurate.

The discharge of untreated or treated wastewater into inland surface water will bring pharmaceutical residues to freshwater, which in many places are used as raw water in waterworks. Based on the current low concentrations of carbamazepine and other pharmaceutical residues in drinking water, WHO has concluded, that “appreciable adverse impacts on human health from drinking water are very unlikely”.

Tests with advanced methods in pilot scale, at four regular WWTPs, showed that it was possible to remove pharmaceutical residues from regular effluent to a high degree, under different conditions in regular plant layout, process performance and different concentrations of pharmaceutical residues. The WWTPs had major differences in 1) sludge age 2) specific flow of wastewater and share of stormwater 3) specific consumption of API. 4) wastewater temperature and 5) hydraulic retention time, especially in the biological treatment.

In the effluents of the four WWTPs, 22-26 of the originally selected 93-97 APIs were regularly quantified, which reduced the evaluation set, of mainly bioactive APIs, to 22-26 APIs, which were evaluated during the pilot tests. The dilution factors in the receiving water, for the effluent wastewater from the four WWTPs, were sufficiently large to dilute all APIs in the effluents below the predicted critical environmental concentration (CEC) for bioaccumulation. This result highlights that the release of the examined APIs into these receiving waters, is not critical today, regarding aquatic concentrations.

The designed stationary and mobile pilot plants, with nanofiltration, activated carbon and ozonation, worked well after minor adjustments. The only complement installed in the mobile pilot plant was the addition of sand filters, to remove suspended solids from the regular effluents at Uppsala and Västerås WWTPs. Henriksdal and Käppala WWTPs have already full-scale sand filters installed. The installed sand filters prolonged the hydraulic operation time of the GAC filters between backwashes, due to a lower load of particles, that would otherwise have clogged the GAC filters. An alternative measure to increase the hydraulic capacity of the GAC filter, was tested by changing GAC, to a product with larger grains. The hydraulic capacity increased significantly after the exchange, but the chosen product showed to have poor adsorption capacity of APIs.
In the pilot tests with nanofiltration, removal efficiencies of APIs varied between 38-99%, with a median removal efficiency of 90% for the 32 APIs. The total volume reduction (VRF) of the polluted hydraulic flow was 20, resulting in a retentate stream of 5%, that must be further treated. The removal efficiency of APIs by nanofiltration could have been higher than 90%, if the specification of the applied membrane had been correct. The molecular weight cut off for the applied membranes showed to be twice the value declared, 300 instead of 150 Da. To better understand and predict the removal of APIs in nanofiltration, a model was developed with the PLS methodology, to predict the rejection in a nanofilter plant, treating effluent municipal wastewater. In the development of the model, 59 physicochemical properties were reduced by multivariate analysis to four descriptive parameters. To predict the rejection of an API, four plant specific constants must be determined, which are then multiplied with the four identified parameters: polarizability, sphericity, charge and the ratio of hydrophobic and polar accessible surface area of the molecule.

The pilot tests with ozonation, operated with ozone doses of 5-7 g/m$^3$, reached 85-95% overall removal efficiency of APIs. Out of 26 APIs, 20 APIs were removed by 80% or more by ozonation. Fluconazol and bupropion were the most resistant APIs to ozonation. Biomarker responses in the ozonated wastewater were lower than the responses in regular effluent. The effluent standards of pharmaceutical residues will influence the applied ozone dose, but to high doses can have adverse effects on aquatic organisms. The working environment for the operators is important to protect from ozone residues and leaks from the dosing system. During the pilot tests, an ozone detector monitored the ambient ozone concentration, ready to stop the ozonation in case of leakage.

In the pilot tests with activated carbon in form of GAC and PAC, 95% removal efficiency was reached for almost all tested pharmaceutical substances. PAC had generally higher removal efficiency of APIs, at a lower carbon usage rate than GAC, but well-performing GAC products showed to approach the performance of PAC. The fresh PAC dose was reduced by the recirculation of used PAC. Depending on the effluent standards and PAC product, the contact time and PAC dose can be varied to achieve different degrees of removal, both with respect to the overall pharmaceutical removal and with respect to individual substances. Memantine, fluconazole, irbesartan and clindamycin were the poorest removed APIs by activated carbon. Biomarker responses to
unspecified chemicals were reduced in fish exposed to GAC treated effluent. PAC-effluents were not subject for biotests.

An important finding from the present study is the large variations in specific carbon usage rates between GAC and PAC products and between individual APIs. A high consumption rate will increase the used amount of activated carbon, shorten the operation time between exchange of GAC, increase the ecological footprint and the running cost. Concerning GAC, evaluation of products with respect to hydraulic capacity is also important. In conclusion, screening of different GAC or PAC products should be done before acquisition to a specific WWTP.

The implementation of PAC in existing WWTPs is probably the fastest and most cost-effective solution, when the existing active sludge step can be used. This work raised the awareness of the necessity of a separate PAC step at WWTPs in Sweden and other countries, where the biological excess sludge is, to different extent, used in agriculture, after sludge stabilization. A dosing of PAC in the biological step would enrich APIs in the sludge and thereby ruling out the recirculation of sludge from WWTPs to agriculture. The pilot tests showed that ozonation, GAC and PAC all performed well at the different WWTPs visited and the processes can be chosen independently of wastewater matrix.

The removal efficiencies varied for different APIs and different treatment methods. In most cases, a PAC system was the broadest working removal process to achieve very high removal efficiencies. The effluent standards of pharmaceutical residues will influence the selection of treatment method, since the removal efficiencies differ for the individual APIs.
5 Conclusion, future perspective and recommendations

The overall aim of the present work was to provide the appropriate knowledge enabling the WWTPs to remove pharmaceutical residues according to the demand of legislation and according to the present scientific and technical frontline.

Selected biomarkers in fish, exposed to regular WWTP effluent, showed effects on biological systems for degradation of harmful substances i.e. detoxification in exposed fish. The work showed that some pharmaceutical residues are slowly degraded and widespread in the aquatic environment.

Potential technologies for removal of pharmaceuticals were selected and tested in pilot scale: nanofiltration, GAC and PAC treatment and ozonation. In the pilot studies, each tested technology removed more than 80% of the APIs on average. To reach more than 90% removal, activated carbon is considered to be the first option with a necessity of screening for appropriate PAC and GAC products before purchase.

The combination of chemical analysis of APIs, together with monitoring of selected biomarkers in fish, was useful for the evaluation of treatment technologies for removal of pharmaceutical residues. Ozonation and GAC filtration significantly decreased both the numbers and the concentrations of APIs present in regular effluent water, as well as biomarker responses to unspecified chemicals in fish, exposed to ozonated or GAC-filtered effluent.

Under present circumstances, ozonation or adsorption onto activated carbon were thus shown to be the main candidate technologies to increase the removal of pharmaceutical residues from municipal wastewater.

The removal of pharmaceutical residues is considered today as the latest launched addition to municipal WWTPs, but it will not be the last improvement, in the still on-going development of water and wastewater management, that started 6 000 years ago, as described in the introduction to this thesis.

Future perspective and recommendations

Future research and development regarding pharmaceutical residues in wastewater should include extended removal tests in pilot and full-scale plants and modelling of the removal processes and mechanisms.
Furthermore, additional long-term biotests should be undertaken in combination with future pilot and full-scale tests. The complementary pilot tests should include trials with control strategies for the dosing of ozone and PAC respectively to the effluent wastewater. Input parameters to the control strategies must reflect the content of dissolved and adsorbing organic substances, but also the content and distribution of particles in the effluent wastewater. All control parameters must preferable be measured on-line. Measurements of ozone in the off-gas can also be used in a feed-back control strategy of the ozone dose.

The complementary biotests should involve additional species and end-points, but also include studies in situ of aquatic organisms and the associated food web in the receiving waters of WWTPs. Today short term biotests, reflecting acute toxicity are performed, but long-term tests to study chronic effects or chronic toxicity from regular and advanced treated effluent, by ozonation and activated carbon, should also be undertaken.

Furthermore, the commercial chemical analyses of pharmaceuticals in wastewater, and in sludge particular, need to be improved to reach lower detection limits. Additionally, it is desirable that these analyses can be offered to a lower cost than today. The high cost limits the monitoring of WWTPs, but also the number of samples and thereby the accuracy of lab and pilot studies. Alternatively, a subset of indicator APIs can be selected and offered to the market at lower cost.

Sweden’s first full-scale ozonation plant for removal of APIs was designed and operated at Knivsta WWTP. The design was done by the author and was based on the findings from the ozonation pilot tests of this study. Some knowledge gaps for future studies were identified:

1) Strategies for the distribution of ozone to different zones in the contact tanks.

2) Best solution for quenching surplus ozone and degradation of potential by-products from ozonation - is the standard sand filter the best alternative or can other type of filters lower the costs or improve the removal?

3) Up-scaling from pilot scale to full scale – is the first approach to maintain the same water depth and hydraulic retention time correct?
In Sweden, advanced treatment at WWTP is discussed in the wastewater sector and political initiatives are taken to facilitate the developing of know-how, ahead of a potential implementation in full-scale plants. In this process, the author wants to give the following recommendations to the organizations dealing with removal of pharmaceutical residues:

1) Increase the understanding of the present situation in the WWTP with respect of the mass flow of specific pharmaceutical residues, by measurement of influent and effluent flows. To perform this mapping, there is a need of external access to advanced analysis of a multitude of substances, present at very low concentration levels. This is a considerable problem today, due to few available laboratories and high cost.

2) Evaluate the collected data with respect to Environmental Quality Standards (EQSs) and the EU Water Frame Directive (WFD), but to provide a better foresight with respect to future regulatory initiatives, results from the research frontier in the area must be taken into account. For this to be successful, a discussion with representatives from academy and wastewater organizations is crucial. There is a need for development and application of less labor intensive ecotoxicity tests to monitor also long-term effects.

3) Evaluate the present plant treatment technology performance versus new requirements. Are process changes in the existing plant sufficient to fulfill new requirements? How can additional technologies improve the removal and is it possible to install them physically on the plant? Preferable, the proposed technology must be tested at site.

4) Implement appropriate advanced treatment. Aim to install a flexible technology, which works as broad and efficient as possible. The selected technology must be adjustable to compensate for variation in pollution load. Taking into account the consumption of resources, and the production of by-products, that must be further processed.

Internationally, development and improvement of methods for removal of pharmaceuticals are ongoing as well. A continuation and extension of the international collaboration is valuable and desirable.
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