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Jesper Svanfelt

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Jesper Svanfelt

Laboratoriet för organisk kemi
Institutionen för naturvetenskaper
Åbo Akademi
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OCCURRENCE AND PHOTOCHEMICAL FATE OF SELECTED PHARMACEUTICAL ACTIVE INGREDIENTS IN THE AQUATIC ENVIRONMENT

Preface

This work was carried out at the Laboratory of Organic Chemistry, Department of Natural Sciences, Åbo Akademi University, during the period 2007-2010. The work was financially supported by The Finnish Graduate School of Environmental Science and Technology, Walter och Lisi Wahls stiftelse för naturvetenskaplig forskning, Åbo Akademi Research Foundation and Ålands självstyrelses 75-årsjubileumsfond.

I would like to express my sincerest gratitude to my supervisor, Professor Leif Kronberg, for the many laughs he has contributed with over the years, for his patience with my slow writing and for his expert advise. PhD Johan Eriksson (Stockholm University) is also gratefully acknowledged for a good collaboration and photochemical expertise. I also wish to thank PhD Atlasi Daneshvar (Swedish University of Agriculture) for a successful collaboration. Furthermore I wish to thank Professor Marja-Liisa Riekkola and Professor Christian Zwiener for reviewing my thesis and Åke Bergman for accepting to be my opponent.

Special thanks go to my close family for their support and love. Last but not least, I wish to thank my wonderful wife Linda-Marie for her everlasting understanding, support and friendship.

Mariehamn, 2012

Jesper Svanfelt

Abstract

Over the past few decades drugs have become an important part of our everyday life and, consequently, they are consumed in very large quantities every year. The active ingredients of drugs are excreted from the body via urine and feces and carried to wastewater treatment plants. As complete removal in the treatment plant is seldom achieved, these biologically active compounds are continuously discharged to the aquatic environment with the effluent water. Recent developments in analytical chemistry have enabled reliable quantification and monitoring of a range of active pharmaceutical ingredients in several environmental matrices. Currently, studies on the environmental fate of drugs are receiving increasing attention, as this is an important research area of which there is still very limited knowledge.

This thesis focuses on the environmental occurrence and photochemical fate of selected active pharmaceutical ingredients. Analytical methodologies for quantification of pharmaceuticals in wastewater and surface water and for studying photochemical transformation pathways of individual compounds are presented. In addition, some synthetic methods for accessing transformation products of the anti-inflammatory drug diclofenac are described.

In conclusion, this thesis contributes with new and important knowledge that will be helpful for the environmental risk assessment of the studied drugs.

Abstrakt

Under de senaste årtiondena har läkemedel blivit en allt viktigare del av vår vardag och följaktligen konsumeras de årligen i enorma mängder. Läkemedlens aktiva ämnen utsöndras från kroppen med urin och fekalier, varefter de transporteras till avloppsreningsverk. Till följd av att reningsverken inte fullständigt förmår rena avloppsvattnet från dessa biologiskt aktiva ämnen kommer de kontinuerligt att tillföras vattenmiljön med utloppsvattnet. Den senaste tidens utveckling inom analytisk kemi har gjort det möjligt att, på ett tillförlitligt sätt, kvantifiera och studera ett stort antal aktiva ämnen i ett flertal olika provtagningsmiljöer. Idag finns ett ökat forskningsintresse kring de aktiva ämnenas öde i miljön, d.v.s. hur de påverkas av olika fysikaliska, kemiska och biologiska processer i naturen, eftersom detta är ett viktigt forsningsområde som det än så länge finns mycket lite kunskap om.

Denna avhandling inriktar sig på de aktiva ämnenas förekomst och fotokemiska öde i miljön. I avhandlingen presenteras framtagna analytiska metoder för kvantifiering av medicinska föreningar i avloppsvatten och ytvatten samt för studier av vissa utvalda föreningars fotokemiska transformationsvägar. Därtill beskrivs några syntetiska metoder för att få tillgång till transformationsprodukter av den antiinflammatoriska substansen diklofenak.

Sammanfattningsvis tillför avhandlingen ny och viktig kunskap som kommer att vara till hjälp vid miljöriskbedömning av de studerade medicinska föreningarna.

List of publications

I Analysis of thyroid hormones in raw and treated waste water. Svanfelt, J., Eriksson, J., Kronberg, L. Journal of Chromatography A **2010**, 1217, 6469–6474.

II Neglected sources of pharmaceuticals–footprints of a reggae festival. Daneshvar, A., Svanfelt, J., Kronberg, L., Weyhenmeyer, G. Journal of Environmental monitoring **2012**, 14, 596–603.

III A Photochemical study of diclofenac and its major transformation products. Eriksson, J., Svanfelt, J., Kronberg, L. Photochemistry and Photobiology **2010**, 86, 528–532.

IV Photochemical transformation of the thyroid hormone levothyroxine in aqueous solution. Svanfelt, J., Eriksson, J., Kronberg, L. Environmental Science and Pollution Research **2011**, 18, 871–876.

V Synthesis of substituted diphenylamines and carbazoles: phototransformation products of diclofenac. Svanfelt J., Kronberg, L. Environmental Chemistry Letters, **2011**, 9, 141–144.

Other publications

VI Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river–lake system. Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G. Chemosphere **2010**, 80, 301–309.

VII Winter accumulation of acidic pharmaceuticals in a Swedish river. Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G. Environmental Science and Pollution Research **2009**, 17, 908–916.

VIII Environmental Fate and Hazards of the Pharmaceutical Diclofenac in Aquatic Environments. Svanfelt, J., Kallio, J-M., Eriksson, J., Kronberg, L**.** Chapter 11 (243–255) in**:** Contaminants of emerging concern in the environment: Ecological and human health considerations. Halden, R. (editor), American Chemical Society **2010**.

Jesper Svanfelt has contributed to the papers in this thesis as stated below:

I The respondent was involved in sample collection, method development and analysis. He was also mainly responsible for writing the paper.

II The respondent participated in the analytical work and data interpretation and was involved in writing the paper.

III The respondent participated in the analytical work and data interpretation and was involved in writing the paper.

IV The respondent was involved in sample analysis and data interpretation and was mainly responsible for writing the paper.

V The respondent performed the synthetical work, analyses and data interpretation. He was also mainly responsible for writing the paper.

List of Abbreviations

AI	active ingredient; the chemical compound responsible for the biological activity of a drug
API	atmospheric pressure ionization
Da	Dalton, unified atomic mass unit
ESI	electrospray ionization
FRET	Förster resonance energy
GC	gas chromatography
HLB	hydrophilic lipophilic balance
HOMO	highest occupied molecular orbital
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IS	internal standard
LAH	lithium aluminum hydride
LC	liquid chromatography
LUMO	lowest unoccupied molecular orbital
MAOS	microwave assisted organic synthesis
MRM	multiple reaction monitoring
MS	mass spectrometry
MS/MS	tandem mass spectrometry
m/z	mass to charge ratio

NMR	nuclear magnetic resonance
NSAID	non-steroidal anti-inflammatory drug
PEC	predicted environmental concentration
PET	photo-induced electron transfer
PTP	phototransformation product
Q	quadrupole
qMS	single quadrupole mass spectrometry
r-T_3	(2S)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3-iodophenyl]propanoic acid or 3,3',5'-triiodo-L-thyronine
SPE	solid phase extraction
3,3'-T_2	2-amino-3-[4-(4-hydroxy-3-iodo-phenoxy)-3-iodo-phenyl]propanoic acid or 3,3'-diiodothyronine
3,5-T_2	2-amino-3-[4-(4-hydroxyphenoxy)-3,5-diiodophenyl]propanoic acid or 3,5-diiodothyronine
T_3	(2S)-2-amino-3-[4-(4-hydroxy-3-iodo-phenoxy)-3,5-diiodo-phenyl]propanoic acid or 3,3',5-triiodo-L-thyronine
T_4	(2S)-2-amino-3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl]propanoic acid or 3,5,3',5'-tetraiodo-L-thyronine
TOF	time of flight
TP	transformation product
WWTP	wastewater treatment plant
UV	ultra violet

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Introduction

Background

It is safe to assume that drugs have been present in the environment for as long as people have consumed them, yet they were only recently detected. Very little is known about the fates and long-term effects of the active ingredients (AIs) found in the environment to present date, hence they are still widely referred to as “emerging" contaminants. AIs are excreted in urine and feces either as the parent compounds or as metabolites. Modern wastewater treatment plants (WWTPs) are unable to completely remove all AIs from the influent water and as a result many of them are frequently encountered in the effluent and downstream watercourse.

Although some early reports on the occurrence of natural and synthetic estrogens and other AIs in the effluents of WWTPs appeared already during the 60s and 70s (Stumm-Zollinger 1965; Tabak 1970; Garrison 1975; Hignite 1977), it was not until the late 1990s and early 2000s that studies on the environmental occurrence of AIs really intensified (e.g. (Halling-Sørensen B. 1998; Ternes 1998; Kolpin 2002; Vieno 2007). The sudden increase in the number of monitoring studies emerging during this period is correlated to breakthroughs in the development of analytical techniques at that time (Barceló 2007). One particularly important technique was the liquid chromatography-electrospray ionization-quadrupole mass spectrometry (LC-ESI-qMS) coupling, which had a revolutionizing impact on analytical science. Today the ever growing multi-billion dollar pharmaceutical industry in combination with improved analytical and sample preparation techniques have led to the detection of more than 180 AIs in environmental samples (Sadezky 2010), and there is now a shared concern among expert stakeholders worldwide about the potentially damaging impact of pharmaceuticals on aquatic ecosystems (Doerr-MacEwen 2006).

In the environment AIs may interact with all components in the ecosystem, both abiotic and biotic. Some of these interactions may eventually lead to modifications (transformations) of their molecular structures, creating new compounds with different

physicochemical and biological properties. The need to also consider the products of transformation reactions further complicates the environmental risk assessment of AIs. A considerable part of this thesis will be concerned with one of the most important natural transformation processes for a wide range of organic pollutants, namely phototransformation. As most AIs contain functional groups that absorb ultra violet and/or visible light, sunlight-mediated reactions in water may have a decisive impact on their environmental fate.

Reasons for concern

There are four principal reasons why AIs qualify as potent environmental contaminants: a) they are produced in significant quantities; b) they are continuously introduced to the environment via the effluent of WWTPs; c) they are chemically designed to elicit biological responses at low concentrations; and d) they are effectively dispersed in the environment.

WWTPs

Municipal WWTPs are undoubtedly the most important source of AIs in the aquatic environment. However, there are other unexpected sources as well, which may even become temporary 'hot spots' of AIs. An example of such a case is the reggae festival that will be discussed later. A typical treatment process in a WWTP consists of several steps e.g.: 1) preliminary treatment; 2) primary treatment; 3) advanced primary treatment; 4) secondary treatment and 5) tertiary treatment. The purpose of preliminary treatment is to remove large objects from the water that would otherwise disturb the rest of the treatment process. During steps 2) and 3) suspended solids are removed from the influent using methods such as screening, filtration, sedimentation, aeration and flotation. The water is then subjected to microbial degradation, which removes suspended and dissolved organic matter (step 4). Nutrients (phosphorous and nitrogen) are often removed during this stage (step 4) as well. Finally, the effluent is chemically disinfected, usually by addition of sodium hypochlorite or through ozonation (step 5), before discharged into the recipient water. Depending on the disinfection

method used an additional step may sometimes be required to remove residual disinfectant. An alternative and increasingly applied technology for effluent disinfection is UV irradiation (Canonica 2008). The main benefit of this technique over chemical disinfection methods is that no subsequent treatment is required for removal or inactivation of residual disinfectant (Tchobanoglous 2002; Casson 2006). The challenge, though, is to ensure that all of the water is continuously subjected to a radiation dose that is high enough for complete pathogen elimination (Pilkington 1995). From an environmental viewpoint the increasing application of UV irradiation technology in WWTPs raises concern over the risks of introducing some potentially toxic phototransformation products (PTPs) of photoreactive organic pollutants (e.g. AIs) directly to the aquatic environment (Canonica 2008). Although some water purification techniques such as granular activated carbon are potentially capable of effectively removing AIs and other polar pollutants from the wastewater, it would come at an unsustainably high cost. To reduce environmental contamination it is instead suggested that efforts are put on optimization of available treatment methods as well as reduction at the source (Jones 2005). Another reduction measure often appearing in discussions is connected to the actual preparation of the AIs or, in a larger context, the design trends within the pharmaceutical industry. The green chemistry concept, as defined by Paul Anastas[1], is "the design of chemical products and processes that reduce or eliminate the use and generation of hazardous substances" (Khetan 2007). If adopted by the pharmaceutical industry the green chemistry approach would result in AIs that, due to their chemical design, are more effectively degraded in WWTPs than many of today's drugs.

The continuous environmental input from WWTPs can make compounds, despite being susceptible to natural transformation processes, appear persistent in a confined environment. This so called 'pseudo-persistence', the result of a roughly equal number of molecules entering and leaving a system at any given time, has recently been demonstrated for diclofenac; a common non-steroidal anti-inflammatory drug (NSAID),

[1] Director of Yale University's Center for Green Chemistry and Green Engineering

in a river downstream of a WWTP (Radke 2010). The compound was found to be added to the river at approximately the same rate as it was being photo-transformed. Areas experiencing large variations in sunlight availability and temperature with seasonal shifts (like the Baltic region) will accordingly have periods with significantly altered input-output ratios of AIs. Several studies have also shown that AIs tend to accumulate in rivers and lakes during cold and dark periods of the year, reaching substantially higher concentrations (Vieno 2007; Daneshvar 2009; Daneshvar 2010) than during bright and warm periods. In such cases, accumulation is usually connected to a lower output of AIs due to decreased influence of photo- and biotransformation. There are examples of the opposite scenario as well, i.e. when higher environmental concentrations are observed as a result of season-dependent elevated inputs. AIs following such patterns are e.g. antihistamines used by people suffering from allergic reactions. Typically, a consumption peak of these compounds coincides with the sudden rise in the air's pollen levels during spring (Kosonen 2009). Finally, there are compounds like carbamazepine, which, due to their reluctance to transform under any environmental conditions, may accumulate in receiving waters. Because of its persistence carbamazepine has been proposed as a possible anthropogenic marker in the aquatic environment (Clara 2004).

A potential consequence of the continuous discharge of AIs to rivers, apart from potential toxicological and ecological impacts, is the emergence of antibiotic-resistant bacterial strains. Studies suggest that there is a connection between WWTPs and the selective increase of antibiotic-resistant bacteria as well as the occurrence of multi-drug resistant bacteria in aquatic environments (Guardabassi 1998; Kim 2007; Zhang 2009). This is also something that needs to be considered when assessing ecological and human health risks potentially associated with the environmental occurrence of AIs.

Design and consumption

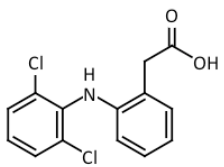
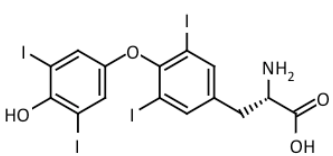
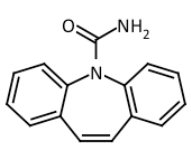
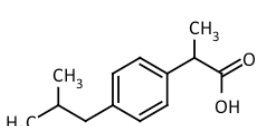
AIs are meticulously designed for optimal interaction with biological systems and are thus potentially hazardous to organisms even at very low levels. Nonetheless, there are to date only two well-documented cases where effects on wildlife have been traced back to specific AIs. These are: the feminization of male fish caused by the synthetic

hormone ethinyl estradiol used in contraceptive pills; and the mass death of Asian vultures caused by veterinary use of the anti-inflammatory drug diclofenac (McGlade 2010). Recently, exposure studies on the NSAIDs diclofenac and naproxen have concluded that both drugs are absorbed, metabolized and accumulated in fish at environmentally relevant conditions (Brozinski 2011; Kallio 2010). Although several other individual AIs are raising concern among scientists, their concentrations in European environments are normally considered too low to independently induce any direct ecotoxicological effects. However, there are approximately 10,000 drugs on the world market today and even in such small countries like Finland yearly consumption rates of common AIs normally exceed several tons (Vieno 2007). This means that in a typical exposure situation in the environment numerous AIs may be found in the same place, in addition to a number of other chemicals, and at the moment there is very limited knowledge about risks from exposure to chemical mixtures of this sort. Although there is an evident need to consider the joint action of AIs in risk assessment strategies, it is simply beyond the capabilities of science to sort out all the interactions that could occur in such a complex chemical cocktail.

Physicochemical properties

In the human body the majority of drugs are absorbed, distributed and excreted by passive diffusion across membranes. In order to afford optimal absorption as well as efficient transportation through the body AIs must possess a dual solubility; part of the molecule needs to be lipophilic enough to penetrate the cell membranes, whereas part of it needs to be water-soluble enough to reach the target area. The water-solubility of AIs is mainly due to the presence of polar, ionizable functional groups (e.g. amino, phenol and carboxylic acid groups) in the molecule. Most AIs are either weak bases or weak acids with their solubility depending on the pK_a of the functional groups within the molecule and the pH of the surroundings. Physicochemical data for some common drugs are presented in table 1. In conclusion, the physicochemical properties of AIs enable them to effectively disperse throughout ecosystems.

Table 1. Physicochemical characteristics of selected AIs.

AI	MW	Structure	Consumption in Finland (kg)	pK_a	$logK_{ow}$	Reference
Diclofenac	296.1		1 000	4.19	1.90	(Melouna 2007) (Vieno 2007)
Levothyroxine	776.7		7	2.40 (COOH) 6.87 (Ph-OH) 9.96 (NH_2)	2.30	(Won 1992) (Norwegian Institute for Water Research (NIVA) 2009) (Svanfelt, 2010)
Carbamazepine	236.3		4 000	13.90	1.51	(Scheytt 2005) (L. A. Viglino 2009) (Vieno 2007)
Ibuprofen	206.3		94 000	4.54	2.48	(Melouna 2007) (Vieno 2007)

Scope of the thesis

The objectives of the thesis were to:

- study sources of AIs in the aquatic environment; determine the occurrence of thyroid hormones in a WWTP effluent, determine the influence of a festival on the occurrence of AIs in a nearby river
- study the aqueous photochemical transformations of selected AIs; establish main reaction scenarios, structurally assign main transformation products and determine the kinetics of main transformation reactions
- get access to reference standards of major transformation products of diclofenac through chemical synthesis

Sample work-up

Solid phase extraction (SPE) is a well-established sample preparation technique that over the past decades has been consistently used in water analysis. It is an effective tool for sample extraction, concentration and cleanup. A range of different sorbents is commercially available and several methods for automated on-line SPE coupled with analytical instruments have also recently been developed (Viglino 2008; Chiuminatto 2010; Trenholm 2009). Solid phase materials with wide extraction abilities, such as Waters Oasis HLB, are frequently used for preparation of complex environmental samples. HLB, which is a hydrophilic-lipophilic-balanced reversed phase sorbent composed of the copolymer [poly(divinylbenzene-co-N-vinylpyrrolidone)] (Figure 1) (Waters Corporation 2002), was used in all of the studies on which this thesis is based. The retention mechanism of HLB sorbents follows the principals of reversed phase chromatography, i.e. analytes in the mobile phase are retained on the solid phase through hydrophobic interactions (nonpolar-nonpolar interactions and van der Waals forces).

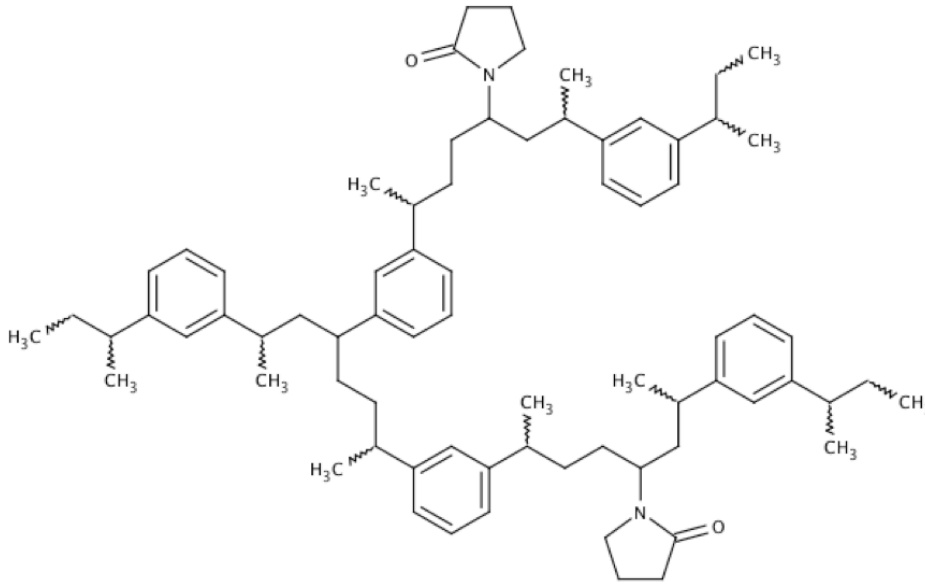


Figure 1. Chemical structure of the Oasis HLB solid phase extraction sorbent [poly(divinylbenzene-co-N-vinylpyrrolidone)].

Analytical tools

Liquid chromatography

A key step in the analysis of complex environmental samples is to achieve efficient separation of the sample components before introducing them to the analytical instrument. Liquid chromatography (LC) is a useful technique by which sample molecules or ions dissolved in a solvent (mobile phase) are separated as a result of their unequal interactions with the stationary phase (solid). The stationary phase is normally contained within a column through which the mobile phase is passed and the analytes should travel through the column with the mobile phase at different rates. Optimum selectivity with LC is dependent on the selection of an appropriate separation mode, stationary phase structure and mobile phase composition. Chemical compounds are separated on the basis of three primary chemical characteristics, i.e.: polarity, electrical

charge and molecular size. The most common separation mode, as far as organic molecules are concerned, is polarity-based, i.e. analytes are separated due to differences in polarity, which in turn is dependent on the nature and number of the functional groups they contain. In order to afford separation the polarity of the mobile phase and stationary phase must differ. In normal phase LC the stationary phase is composed of a polar, solid material (usually silica gel) and the mobile phase is a nonpolar solvent mixture. The activity or polarity of the stationary phase is easily modified by chemically bonding less polar functional groups to the silica surface. In reversed phase LC (RPLC) the stationary phase is nonpolar and the mobile phase is polar. Common stationary phase materials in RPLC (in order of decreasing polarity) are composed of cyanopropylsilyl- [CN], n-octylsilyl- [C8] and n-octadecylsilyl- [C18] moieties on silica. Phenyl columns are also commonly used as alternatives to C18. These columns enable π-interactions between the analytes and the stationary phase, which may influence the chromatographic behavior of some analytes.

RPLC is today without a doubt the most utilized separation technique for environmental analysis of polar organic pollutants. Pharmaceuticals and pharmaceutical residues found in nature are normally quite hydrophilic and not volatile enough for gas chromatographic (GC) separation. Thus most reported GC methods tend to offer limited applicability and often require time-consuming derivatization steps. The high pressure liquid chromatographs of today, with automated sample introduction, have markedly simplified the analytical procedure and the combination with mass analyzers have allowed for rapid and reliable analysis of highly complex environmental and biological samples.

Liquid chromatography – mass spectrometry

Electrospray ionization

The evolvement of atmospheric pressure ionization (API) techniques during the 1990s was crucial for enabling the combination of LC and mass spectrometry (LC-MS). Until then, the LC-MS coupling had been restricted by the inability of previous ionization

techniques to handle the continuous flow of the LC. Today the LC-MS system has become one of the most powerful analytical tools for organic compound analysis. The soft ionization process provided by the API generally affords a mass spectrum dominated by a single ion, corresponding to the molecular weight of the protonated $(M+H)^+$ or deprotonated $(M-H)^-$ molecular ion. One API-technique frequently used in connection with organic compound analysis is electrospray ionization (ESI). ESI allows ionic species in solution to be analyzed by mass spectrometry by using electrical energy to assist the transfer of ions from solution to gas phase. ESI is one of the softest available ionization techniques, which means there is little risk of in-source fragmentation of molecular ions. Due to the ability of ESI to form multiply charged ions from high molecular weight compounds, it allows the masses of very large compounds to be determined on instruments with a limited mass range. It was this unique property that in the beginning narrowed its application to analysis of proteins and polymers. However, ESI was later found to provide highly sensitive analysis of small polar organic molecules, which, in addition to its easy application to high-pressure LC systems, made it a popular technique in the field of environmental science. The mechanism of ESI is illustrated and described in Figure 2.

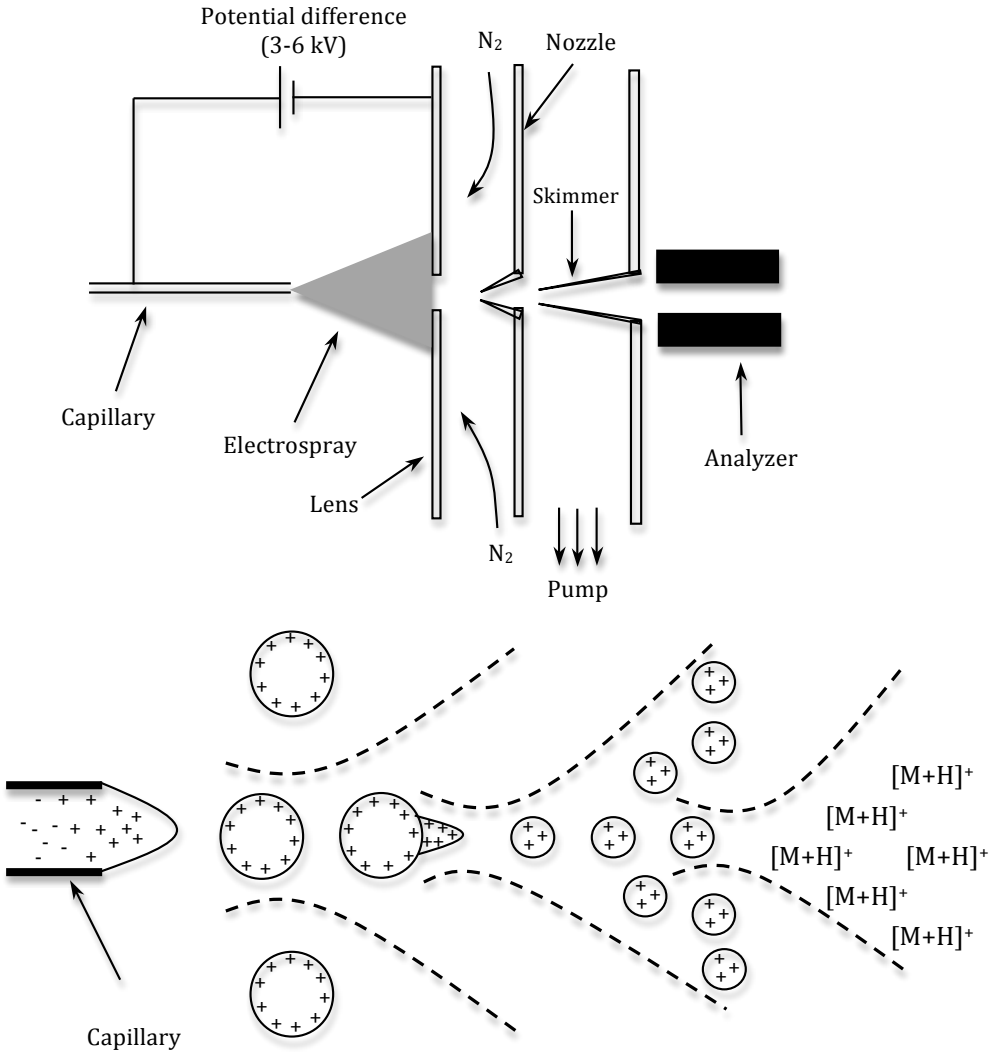


Figure 2. The principles of ESI. Liquid exiting the capillary tube is subjected to a strong electric field, which causes charge accumulation at the surface. Eventually the liquid surface will brake into highly charged droplets with the same polarity as the capillary voltage. The remaining solvent molecules in the droplets are evaporated off by the aid of heated nitrogen gas and the molecular ions, usually in the form of $(M+H)^+$ or $(M-H)^-$ depending on the mode of ionization, are transferred into the high vacuum of the mass analyzer.

Mass analyzers

The mass analyzer separates the gas phase ions produced by the ion source according to their mass-to-charge ratios (m/z). There are different principles by which this separation can be achieved and, consequently, there are different types of mass analyzers available, each of them best suited for different purposes. Analyzers frequently used in the analysis of environmental samples are: quadrupole (Q), time of flight (TOF) and ion trap (IT) instruments. Sequential combinations of analyzers, with the purpose of increasing instrument versatility, are also common. Notable combinations are e.g. the triple quadrupole (QqQ), quadrupole time-of-flight (Qq-TOF) and hybrid triple quadrupole linear IT (QqLIT) mass analyzers. Quadrupole instruments are composed of four parallell cylindrical or hyperbolic rods according to Figure 3.

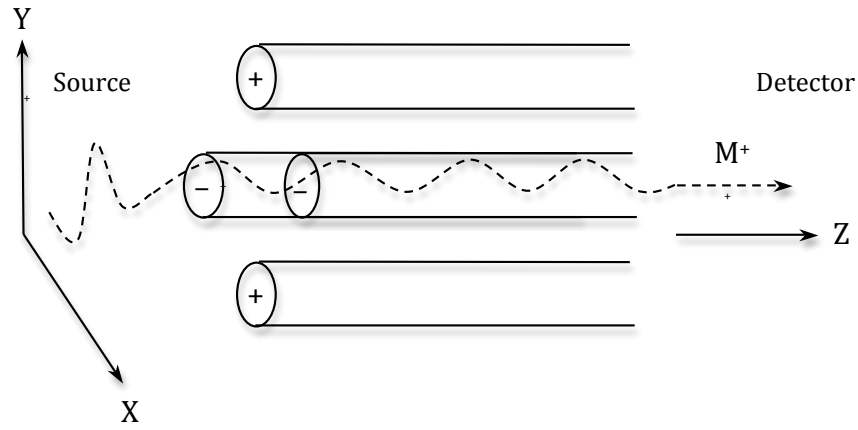


Figure 3. An ion entering the space between the rods is exposed to oscillating electric fields. Positive ions are attracted to the negative rods and if the potential changes sign before the ion collides with the surface of the rod the ion will change its direction. Thus only ions with a specific m/z will reach the detector while those with unstable trajectories will collide with the rods.

Triple quadrupole analyzers may be utilized for multiple purposes depending on how Q_1 and Q_2 are set to operate (Figure 4). During *product ion scan* only ions of a specific m/z are transmitted from Q_1 to q while Q_2 is set to scan an entire range of m/z, providing information on possible product ions. In the *precursor ion scan* mode the precursor ions are scanned in Q_1 and a specific product ion is selected in Q_2. This enables all precursor ions that produce a specific product ion upon collisions in q to be detected. Both Q_1 and Q_2 may be scanned together with a constant mass offset between the two, i.e. with a preset mass difference of *x* detection will occur only if ions of mass *m* transmitted from Q_1 produce product ions of mass *m–x* in *q*. This is called *neutral loss scan*. The operating mode that makes triple quadrupole analyzers exceptional instruments for highly sensitive target compound analysis is the SRM or MRM mode (single reaction monitoring or multiple reaction monitoring). In the SRM mode both Q_1 and Q_2 are set to transmit a specific m/z, i.e. a specific precursor – product ion transition is monitored. This increases both sensitivity and reliability of the analysis. MRM enables multiple transitions to be monitored simultaneously, which is particularly useful when a number of compounds need to be processed in a single run. LC-ESI-QqQ MS combinations are frequently used for quantitative analysis of environmental organic pollutants as the instruments usually provide very low limits of quantification (LOQ) when operating in the SRM or MRM mode.

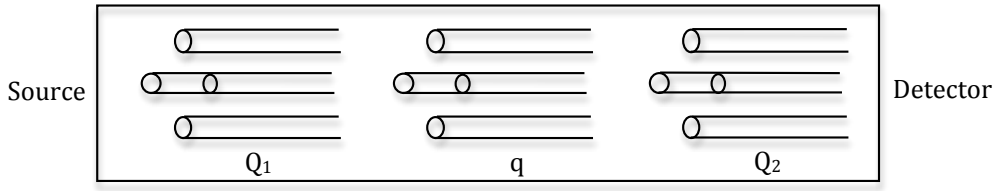


Figure 4. A triple quadrupole instrument consists of three quadrupoles connected in series. This combination allows two MS experiments (MS/MS) to be performed during a single run. Q_1 is a mass spectrometer that may be programmed to transmit only ions of a certain m/z or range of m/z to the collision cell *q*. In q a collision gas is introduced at a pressure that ensures collisions with ions from Q_1. Q_2 is also a mass spectrometer, acting as a second mass filter before the detector.

The QqTOF instrument is a QqQ with the last quadrupole MS replaced by a TOF-MS. The TOF operates by determining the m/z of an ion by measuring its time of arrival at the detector. Ions, initially accelerated by a constant electric field, gain different velocities depending on their m/z and consequently arrive at the detector at different times. QqTOF analyzers combine the high mass resolution and accuracy of TOF MS with the high sensitivity achieved in the MS/MS mode of quadrupole instruments. The more specialized operating (scan) modes of QqQ, such as the MRM mode, are not as efficient on QqTOF instruments and thus the QqQs still remain the instruments of choice for quantitative target compound analysis. However, obtaining accurate masses of both precursor and product ions, which subsequently enables determination of elemental compositions, is a property that makes QqTOF instruments unique. The coupling of an API source (e.g. ESI) to a TOF analyzer was for a long time a difficult task due to the incompatibility of the continuous ion beam generated by the ESI and the pulsed operating process of the TOF. The technique that overcame this problem was orthogonal injection (orthogonal acceleration), which was initially developed in the 1960s but reinvented in the beginning of the 1990s. The LC-ESI-QqTOF MS combination is today increasingly employed in environmental analysis and is a particularly important

tool for the identification of unknown transformation products of organic pollutants, such as the phototransformation products of pharmaceuticals (Petrovic 2007).

IT analyzers use a radio frequency quadrupolar field to trap ions. The ions are trapped in either two or three dimensions, hence the classification into 3D ITs (or quadrupole IT, QIT) or 2D ITs. The main advantages of IT instruments are the high sensitivity obtained in full scan mode and the ability to perform multiple MS-experiments (MS^n). Therefore ITMS is well suited for structural elucidation of unknown compounds. The QqLIT has also recently been successfully applied to the analysis of organic pollutants in wastewater (Martínez Bueno 2009). This instrument, which is basically a QqQ with the last quadrupole replaced by a linear IT, combines the typical operation modes of the QqQ with the highly sensitive full scan mode of the IT.

Photochemistry

Throughout Earth's history photochemistry has played a central role. In fact, it is thought that life originated and evolved as a result of sequences of photochemical reactions (Mauzerall 1990). Solar energy is the most abundant source of energy and ultimately responsible for the most fundamental chemical processes. Radiant energy is usually described in terms of wavelengths, i.e. the shorter the wavelength the more energy it carries (Figure 5). The ultra violet radiation reaching the earth's surface is typically in the 290 to 400nm wavelength range while the maximum intensity lies within the region of visible light (400-700nm). Plants, algae and many species of bacteria make use of the incoming light through photosynthesis, in which electromagnetic energy is transferred into chemical bonds of sugar and oxygen molecules, thereby making it accessible to other life forms.

As AIs typically contain chromophores capable of absorbing sunlight, phototransformation is considered to be an important route of transformation in the environment. UV-irradiation is also increasingly applied in wastewater treatment since it is a particularly effective technique for inactivation of pathogenic microorganisms and

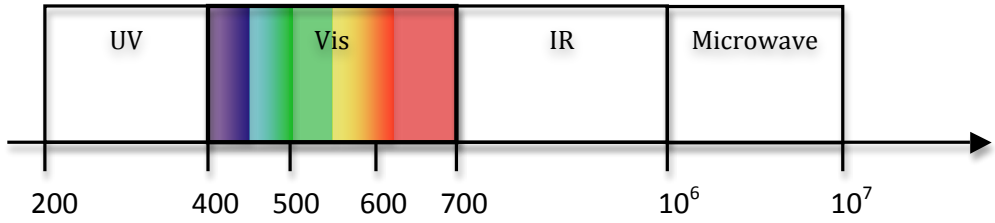


Figure 5. Part of the electromagnetic spectrum.

for disintegration of chemical compounds (Casson 2006; Canonica 2008; Zhou 2001). Photochemical studies of AIs can provide information regarding the identities of major photoproducts and the measured disappearance quantum yields can be used to calculate photochemical reaction rates and half-lives of AIs in surface water.

Activation

In the absence of external energy the electrons in any atom or molecule will occupy orbitals of the lowest possible energy, hence the chemical species will be in its electronic ground state. However, if a suitable amount of energy is applied to the species, e.g. in the form of electromagnetic radiation, the electron distribution may be temporarily disrupted. Absorption of energy can thus force the species to adopt a new electronic configuration, which will be of greater energy than that of the ground state. Any electronic state that is higher in energy than the ground state is said to be excited and it is from these 'activated' states that photochemical reactions arise.

The energies of molecular orbitals are fixed (quantized) and for an electron to move to a higher energy orbital the chemical species must absorb an amount of energy corresponding to the difference in energy between the two orbitals. Energy of light is also quantized and is transferred in the form of photons, which have both wavelike and particle-like properties, each with a specific energy, E, given by Planck's law:

$E = h\nu = hc/\lambda$

where h is Planck's constant, v is the frequency of oscillation, c is the speed of light and λ is the wavelength of oscillation. In addition to electronic excitation there are other types of excitations taking place in the molecule, depending on the energy of the incident radiation. The total energy of a molecule is also comprised of energy due to nuclear motion, i.e. vibrational and rotational energies. Thus the total molecular energy may be written as:

$$E_t = E_e + E_v + E_r$$

where the subscripts refer to total, electronic, vibrational and rotational energy, respectively, and the magnitude of the individual energy contributions are in the order: $E_e > E_v > E_r$. Due to the sizable differences between these energies it is assumed that they can be treated separately.

Absorption of energy quanta corresponding to vibrational or rotational frequencies of a molecule will, accordingly, increase the molecule's vibrational or rotational motion. Such excitations may arise from absorption of longer wavelengths, typically in the infrared region of the electromagnetic spectrum (Figure 5). Electronic excitation through light absorption not only changes the electronic state of the molecule; it changes the vibrational state as well and thus results in an increase in the molecule's vibrational energy level (Figure 6).

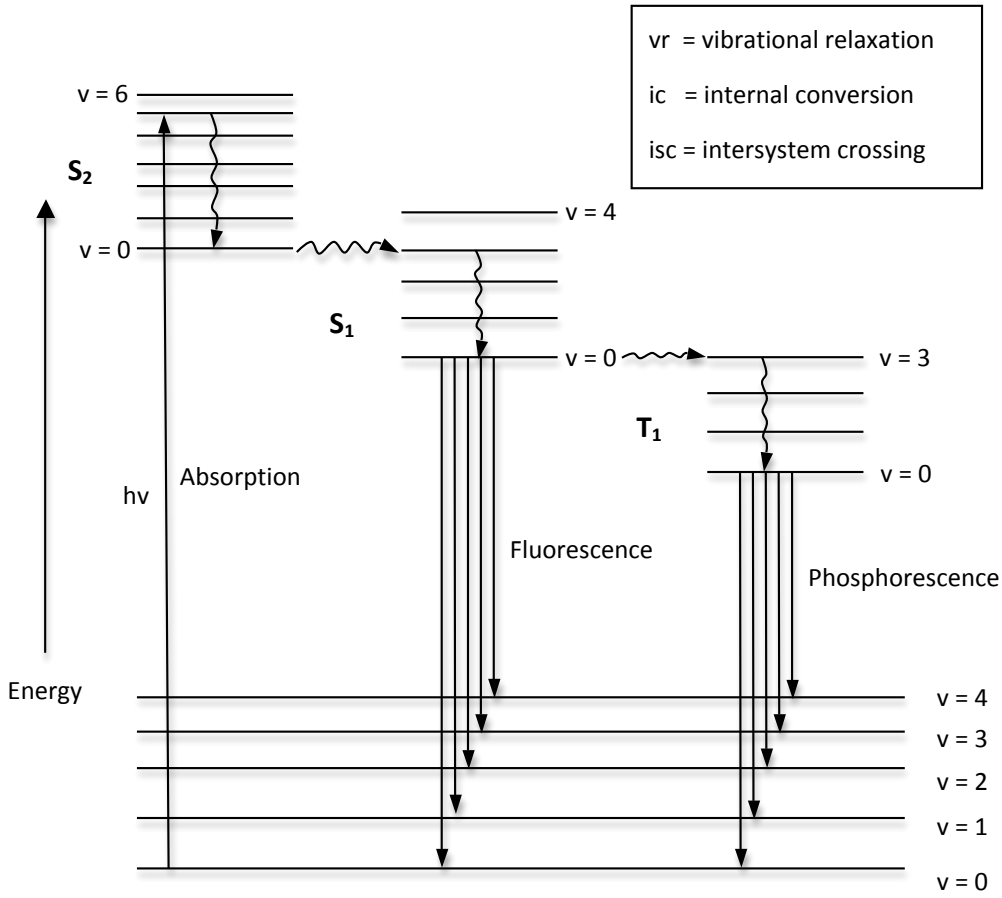


Figure 6. A Jablonski diagram for a hypothetical organic molecule. Adapted from "Principles and Applications of Photochemistry" by B. Wardle, 2009.

In order for an AI to undergo direct phototransformation in the environment it needs to be capable of absorbing wavelengths of > 290nm and the corresponding energies must be enough to afford electronic excitation. The reason why some organic molecules phototransform under environmental conditions and some do not may be found in the structures or, more specifically, in the types of bonds (molecular orbitals) and chemical elements that make up the structures. In a saturated organic compound such as hexane there are only two types of molecular orbitals, bonding and antibonding σ-orbitals (σ

and σ*), with a rather large energy gap between them. On the other hand, in an unsaturated compound like diclofenac there are, aside from σ and σ* orbitals, also bonding and antibonding π-orbitals (π and π*) as well as nonbonding n-orbitals. When present, the n-orbital is often the highest occupied molecular orbital (HOMO) and π* usually the lowest unoccupied molecular orbital (LUMO). Therefore, in compounds containing elements with n-electrons connected to an unsaturated system the lowest energy transitions are normally n → π* (Figure 7). As the energy difference between HOMO and LUMO decreases with an increasing extent of conjugation, the wavelength required for excitation increases. This is why compounds capable of absorbing sunlight normally have highly conjugated π-electron systems. In diclofenac, the combined effects of π-conjugation and the presence of n-eletron containing substituents (N, O and Cl) result in an absorbance that stretches into the sunlight wavelength region.

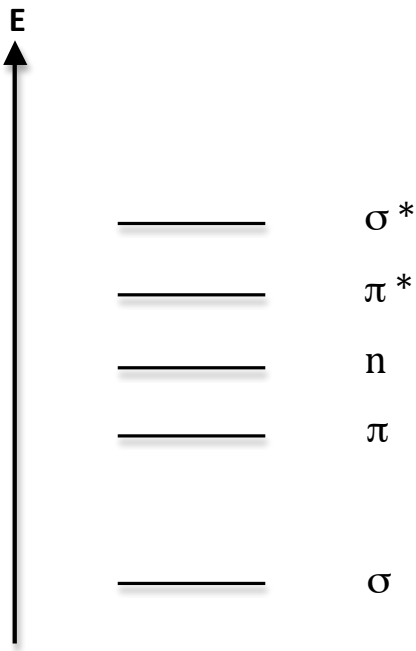


Figure 7. Diagram illustrating relative molecular orbital energies in an organic molecule. Adapted from "Principles and Applications of Photochemistry" by B. Wardle, 2009.

Singlet and triplet excited states

Photochemical reactions usually occur either from the S_1 or T_1 state of the excited reactant molecule. The spin multiplicity is a quantum mechanical concept, which indicates the number of possible quantum states of a system with overall spin quantum number S. The multiplicity is given by the formula 2S + 1, where S is obtained by summation of the spin moments of individual electrons in a species. Since the spin angular momentum of an individual electron is characterized by the quantum number ±1/2 the overall spin (S) of the ground state electrons, where all electrons are spin-paired, is 0. A spin-paired state of a compound is called a singlet state (S) because there is only one orientation in space for such a pair of spins, i.e. 2S +1 =1 (S=∑spin, singlet=0, triplet=1). On the other hand, in a triplet state (T), where two electrons in different

orbitals have parallel spins, the spins may adopt three orientations with respect to an axis and so 2S +1 = 3. Hund's rule states that the lowest energy state is the one with the highest overall spin, the highest multiplicity. Consequently, an excited triplet state will always be lower in energy than the corresponding singlet state (Figure 6).

Information about the properties of excited states may be obtained through studies of absorption and emission spectra. Excited state-lifetimes may be measured directly by monitoring the decay of luminescence and the energy of an excited singlet state may be obtained from the region of overlap of vibrational bands in absorption and fluorescence spectra. For investigation of the nature of reactive states in chemical reactions quenching and sensitization studies may be performed. The addition of a triplet quencher to a photochemical reaction may provide information about the involvement of a triplet mechanism in the reaction as well as the energy and lifetime of the triplet state. Although triplet sensitizers have the opposite effect on triplet state reactions (as compared to triplet quenchers) they may provide similar information.

Fates of activated species

Upon excitation of a molecule a number of processes may take place, some of which are illustrated in the Jablonski diagram (Figure 6). I. *Vibrational relaxation* quickly sets the molecule into the minimum energy structure of the excited state with energy loss to the surroundings (solvent). II. *Intersystem crossing* forms triplet states by spin inversion and vibrational relaxation again enables the energy minimum structure of the new excited state to be reached. III. *Luminescence* (*fluorescence, phosphorescence*) brings the molecule back to its ground state. IV. *Energy transfer* to surrounding molecules (*quenching*) deactivates the molecule. V. *Radiationless deactivation.* Vibrational deactivation returns the molecule to its ground state with the excess energy (heat) expelled to the environment (solvent). VI. *Photochemical reaction*. The excess energy is utilized in bond-breaking and bond-making processes.

Deactivation processes

As mentioned above, an excited molecule may return to its ground state electron configuration through different deactivation processes, which may occur either intra- or intermolecularly.

Intramolecular processes. An excited species may expel the excess energy either by photon emission (luminescence) or by relaxation processes. Fluorescence is the result of a radiative transition from the lowest excited singlet state to the ground state, i.e. S_1 (v = 0) → S_0 + hv. Phosphorescence, on the other hand, involves a spin-forbidden radiative transition from the lowest excited triplet state to the ground state, i.e. T_1 (v = 0) → S_0 + hv (Figure 6). Radiationless transitions include internal conversion and intersystem crossing. Internal conversion involves vibronic transitions between isoenergetic states of the same multiplicity, e.g. S_2 (v = 0) → S_1 (v = 3). Conversion between higher excited states is fast since the energy gap that separates them is small, whereas internal conversion between S_1 and S_0 would be very slow. Intersystem crossing is a transition between isoenergetic states of different multiplicity, e.g. S_1 (v = 0) → T_1 (v = 3), and hence is spin-forbidden.

Intermolecular processes. An excited molecule may give up its excess vibrational energy via collisions with other molecules (e.g. solvent molecules). Such transitions occur between vibrationally excited states (e.g. v = 3) and the v = 0 vibrational level within a given electronic state, e.g. S_2 (v = 3) → S_2 (v = 0) (Figure 6). Electronic energy transfer involves an exchange of excess energy between an excited (donor, D) and a ground state molecule (acceptor A). The transfer may occur either radiatively or nonradiatively. During radiative transfer the donor expels its excess energy in the form of a photon (fluorescence or phosphorescence), which is subsequently absorbed by the acceptor. Thus D is deactivated as A is excited, i.e. D* → D + hv and then hv + A → A*. An obvious criterion for this type of transfer is the overlapping of emission and absorption spectra of the interacting molecules. Förster resonance energy transfer (FRET) is a long-range (10nm distance) energy transfer from D* to A through dipole-dipole coupling, whereas

electron exchange is a transfer process that requires overlap or collision of electron clouds during which electrons are exchanged between molecules. Photoinduced electron transfer (PET) is an electron transfer between an excited and a groundstate species in which either of the two may act as acceptor or donor. The excited species has a vacancy in its HOMO orbital available for accepting an electron as well as a single high-energy electron in its LUMO orbital available for donation. Thus the net result of PET is two charged species: a negatively charged acceptor and a positively charged donor molecule.

Photochemical reactions

If absorption of light by a molecule affords an excited state that is higher in energy than the highest vibrational level (bond dissociation energy) then that particular bond in the molecule will cleave. Photochemical reactions generally take place in one of two ways. During a *concerted process* the product is formed from the excited species in a single step, i.e. electrons are rearranged in a way that causes σ- and π-bonds to simultaneously break and form:

$R^* \rightarrow P$

Alternatively, reactions may take place via *reactive intermediates*, such as radicals. The radical intermediates then react to form products in a secondary process:

$R^* \rightarrow I$ and $I \rightarrow P$

Photochemical reactions may have very different outcomes depending on the nature of the excited state involved and environmental variables, such as solvents and reaction mixture composition. A compound (A) dissolved in a solvent and exposed to radiation at a specific wavelength may predominantly form a singlet excited state which reacts to form rearrangement product B. However, in the presence of a triplet sensitizer the triplet state may be the more populated excited state instead, which results in dissociation products C and D. As previously discussed, an initially formed singlet excited state may prior to reaction partially undergo intersystem crossing to the triplet state,

thus leading to a mixture of products. Photochemical reactions of organic compounds become increasingly complex as the number of chromophores, i.e. the number of reactive centers, in a molecule rise.

Quantum yield

An excited molecule can make use of the excess energy in a productive or unproductive manner. Productive usage results in chemical change through reaction, whereas an unproductive usage deactivates the reactant and returns it to its ground state. The quantum yield is a way of expressing the efficiency of a photochemical process and is defined as:

$$\Phi = \frac{\text{number of molecules undergoing a specific process}}{\text{number of photons absorbed by the photoreactive substance}}$$

The quantum yield is the most important characteristics of a photochemical process and may be defined not only for specific reactions but also for other types of photo-induced processes, e.g. fluorescence and phosphorescence. The quantum yield is normally a number between 0 and 1 and the sum of all individual quantum yields for a specific absorption event should be 1. Exceptions to this statement are e.g. when chain reactions are involved, in which case overall quantum yields may be as large as 10^6.

Chemical synthesis

To be able to carry out reliable screening, quantitation, toxicological and transformation studies on AIs and other pollutants in the environment, reference standards must be accessible. Quantitative analytical methods cannot be developed without access to the target analytes and chemical and biological reactivities are impossible to evaluate in the absence of pure standards. Reference standards of anthropogenic pollutants are normally readily available for environmental chemists, but the transformation products (TPs) of those same chemicals very rarely are. Therefore, following structural

assignment of the TPs of interest, environmental chemists may occasionally need to synthesize the identified compounds to be able to study them further. It is, however, not entirely feasible to study the properties and behaviors of every PTP, as even a single photochemically reactive compound may give rise to a large number of products with significant structural differences, and it would certainly be too time-consuming to synthesize them all. For this reason, initial screening studies are important since they can provide tentative structures of the PTPs and can be used as a basis for picking out the most relevant compounds. In these respects, high resolution and ion trap mass spectrometers are invaluable tools and, in recent years, they have been frequently employed for such purposes (Petrovic 2007; Agüera 2005). Agüera et al. (Agüera 2005) tentatively identified 13 PTPs of diclofenac using both LC-TOF MS and GC-MS instruments and it was this study that prompted us to synthesize some of the compounds, in order to enable further studies on individual PTPs. Some of the compounds described in the study of Agüera et al. have also been proposed elsewhere (Poiger 2001; Moore 1990; Bartels 2007).

Microwave-assisted organic synthesis (MAOS) is a new and somewhat controversial field in chemistry. The poor reputation and skepticism surrounding microwave chemistry during the 1990s, caused by the lack of reaction control and especially the lack of reliable devices for temperature monitoring, was eventually rubbed off around 2000 following the inclusion of MAOS in the pharmaceutical industry. The dielectric heating in microwave ovens is due to two major mechanisms, one of which is dipolar polarization, the other conduction. In order for a substance to be able to generate heat upon microwave irradiation it must possess a dipole moment. When an electric field of microwave frequency is applied the dipole will try to align itself with the oscillating field by rotating. However, the alternating external field and the direction of molecular rotation are not entirely synchronized and, as a result, there will be a phase difference between the orientation of the external field and that of the dipole. The out-of-phase rotating molecules in a liquid are forced quite close to each other and will distribute the gained energy to surrounding molecules via friction and collision, thereby giving rise to

dielectric heating (Lindström 2001). The net effect is thus conversion of electromagnetic energy to heat energy in matter. Conduction is a consequence of the heat generated by the increased number of colliding ions in a solution, which in turn is due to the increased motion of ions upon irradiation (Lindström 2001). The first publications reporting the utilization of microwaves in organic synthesis appeared in 1986 by Gedye et al. (Gedye 1986) and Gigurere et al. (Giguere 1986). Since then, more than 3500 articles have been published in the field (Kappe 2009).

Occurrence of AIs in the aquatic environment (papers I-II)

Occurrence of a thyroid hormone in a WWTP effluent (Paper I)

The aim of this study was to develop a method for quantitation of thyroid hormones in influents and effluents of WWTPs, surface water and tap water. The AI 3,5,3',5'-tetraiodo-L-thyronine (T_4) is a natural hormone secreted from the thyroid gland, which is found in all chordate animals. Synthetic T_4 is used as a medicine in the treatment of hypothyroidsim; a medical condition caused by deficiency of T_4. T_3 or 3,3',5-triiodo-L-thyronine, the primary metabolite of T_4, is even more biologically active and is also used in the treatment of hypothyroidism, although less extensively than T_4. Thyroid hormones serve an important function as they are responsible for regulating the metabolism of all cells in the body. Other metabolites of T_4 are: 3,3',5'-triiodo-L-thyronine (r-T_3), 3,5-diiodothyronine (3,5-T_2) and 3,3'-diiodothyronine (3,3'-T_2). In spite of its low predicted environmental concentration (PEC) T_4 has been recognized as a potential wastewater contaminant of environmental concern (Kostich 2008). High potency compounds, such as hormonal drugs, are capable of disrupting the endocrine system of certain organisms following chronic low-level exposure (Flores-Valverde 2010; Zhang 2004). Therefore, despite their low production volumes compared to some other classes of AIs, the occurrence of these compounds in the effluents of WWTPs needs to be investigated.

T_4 and its metabolites (Table 2) were quantified using a method based on a LC-ESI-QqQ MS instrument coupling. In conjunction with the analytical method a solid phase extraction method for sample concentration was developed. Analyte separation was achieved on a phenyl column that provided high selectivity for the structurally similar analyte molecules, in addition to good peak shapes. The positive ions produced by ESI were analyzed in the MRM mode of a QqQ MS. A parent-daughter transition corresponding to a mass loss of 46 Da was monitored for all compounds. This transition is consistent with an α–cleavage at the amino acid side chain and subsequent formation of the immonium ion $[RCH{=}NH_2]^+$; a characteristic fragmentation behavior of protonated α–amino acids. Some selected method validation parameters for the studied compounds in the different matrices are provided in Table 3.

Table 2. Thyroid hormones included in the LC-MS method.

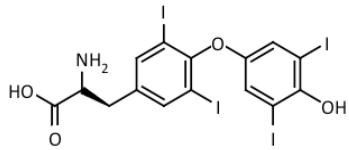
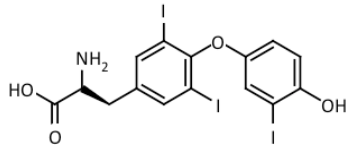
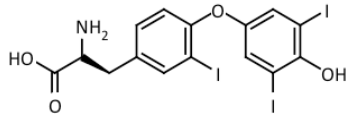
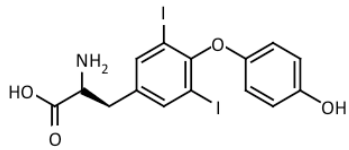
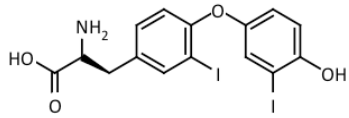
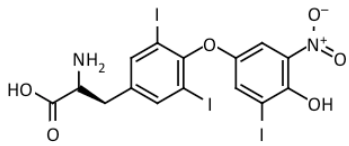
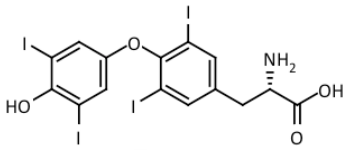
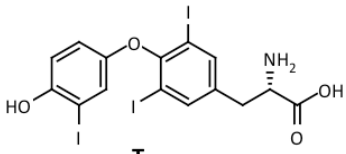
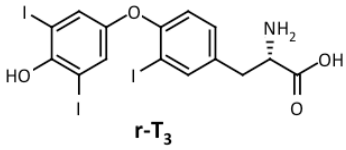
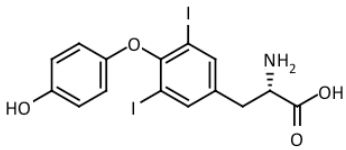
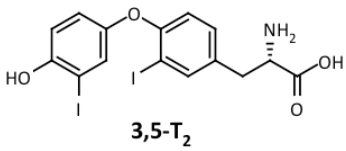
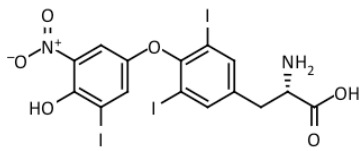
Chemical structure	Molecular weight (g/mole)	Name
	776.7	T_4
	650.8	T_3
	650.8	r-T_3
	524.9	3,5-T_2
	524.9	3,3'-T_2
	695.8	IS

Table 3. Level of quantitation and level of detection in different matrices.

Compound	LOQ/LOD (ngL^{-1})			
	TW	SW	Effluent	Influent
 **T_4**	1.1/0.3	1.9/0.6	5.5/1.6	16.4/4.9
 **T_3**	1.4/0.4	2.8/0.9	6.1/1.9	12.9/4.0
 **r-T_3**	9.9/3.0	10.8/3.2	25.1/7.5	67.6/20.2
 **3,5-T_2**	2.0/0.6	3.0/1.0	6.3/2.0	10.5/3.3
 **3,5-T_2**	13.3/3.9	18.9/5.7	48.3/14.5	84.9/25.6
 **Nitro-T_3** **(IS)**				

Internal standards (IS) are compounds that are chemically and physically similar to the analytes of interest and, if available, (and affordable) stable isotopically labeled analogues are generally preferred. IS are frequently used in the analysis of complex environmental and biological samples in order to correct for systematic errors of the entire analytical method (including sample preparation) and particularly for the *matrix effects* arising during ESI; suppression or enhancement of analyte signals due to co-eluting sample components. ESI is usually more vulnerable to matrix effects as compared to 'harder' ionzation techniques. In this study an IS was synthetically prepared from T_4 by substituting one of the iodines on the phenolic ring with a nitro group. This compound was used as IS for all analytes and provided good to excellent relative recoveries (Table 4).

Table 4. Analyte recoveries in the different matrices. AR = Absolute Recovery; RR = Relative Recovery

Matrix	DOC (mgL^{-1})		Recovery (%)				
			T_4	T_3	r-T_3	3,5-T_2	3,3'-T_2
TW	1.93	AR	38 ± 9	36 ± 5	26 ± 4	22 ± 10	29 ± 7
		RR	95 ± 8	90 ± 3	64 ± 2	54 ± 9	71 ± 5
SW	229.9	AR	21 ± 6	19 ± 1	23 ± 2	15 ± 6	20 ± 5
		RR	67 ± 5	62 ± 7	77 ± 6	48 ± 8	65 ± 11
Effluent	130.5	AR	15 ± 1	17 ± **1**	20 ± 3	14 ± 1	15 ± 4
		RR	50 ± 8	59 ± 10	69 ± 11	47 ± 11	53 ± 13
Influent	60.1	AR	12 ± 2	20 ± 1	19 ± 1	21 ± 2	22 ± 1
		RR	76 ± 6	127 ± 2	117 ± 1	132 ± 7	137 ± 5

Of the studied analytes, only T_4 was detected in the influent and effluent samples of a WWTP in Turku, Finland, at measured concentrations of $64ngL^{-1}$ and $22ngL^{-1}$ for the influent and effluent, respectively. It may thus be concluded that part of the T_4 entering the WWTP will be discharged to the recipient river water via the effluent.

Unexpected sources of AIs (Paper II)

WWTPs are responsible for the continuous input of AIs into the aquatic environment. Still, other sources may unexpectedly emerge as temporary hot spots of AIs. This was

recently shown for an open-air festival arranged annually on the shores of river Fyris in Uppsala, Sweden, which also receives effluent water from Uppsala WWTP. The study was conducted in connection with an ongoing monthly monitoring program, during which the occurrence of neutral, acidic and basic AIs was monitored along the same river between December 2007 and December 2008 (Daneshvar 2010; Daneshvar 2009). The effect of the festival, which annually attracts about 10 000 visitors, on the input of AIs into the river was established by comparison of the results obtained during and shortly after the festival with those collected on a regular basis. In 2008, the measured concentrations of acidic and basic AIs at the upstream sampling site R1 (Figure 8.) 24h after the festival were much higher than during the other 12 sampling occasions.

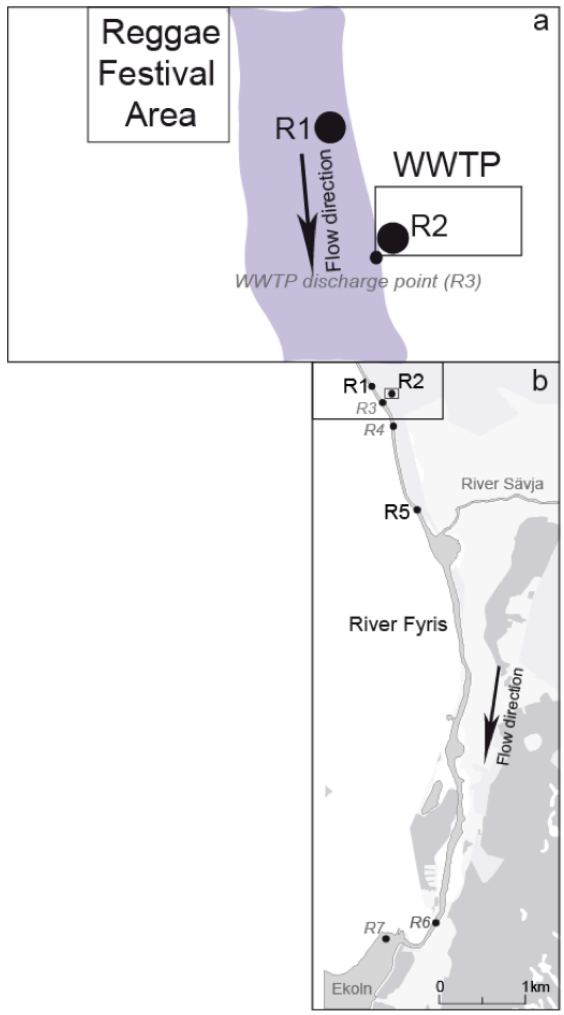


Figure 8. Sampling points along River Fyris. Samples were collected upstream of the wastewater treatment plant (WWTP) in Uppsala (site R1), from the effluent of the WWTP (site R2), and downstream of the Uppsala WWTP (site R5) before, during, and after the 2008 and 2009 Reggae festivals. Reprinted from ”Neglected sources of pharmaceuticals – footprints of a reggae festival”, Daneshvar et al., Journal of Environmental Monitoring, 14 (2012): 296-603.

The concentration peaks of the AIs clearly correlated with peaks in nitrate-nitrogen, ammonium-nitrogen and total nitrogen concentrations, which show that the compounds are excreted with urine and feces. Normally, samples collected upstream of WWTPs are expected to contain much less of the studied AIs compared to samples collected from the effluent. Nevertheless, in this study the mass flows of some AIs at R1 (Figure 9) 24h after the festival clearly exceeded those at the effluent. In 2009, no signs of elevated concentrations of AIs or urine-derived nitrogen could be observed at site R1. This may be due to the different weather conditions during the festival in 2008 and 2009. In 2008 a heavy rainfall event took place the day before sampling whereas very little precipitation occurred in connection with the 2009 festival. Consequently, it is plausible that the AIs were rapidly transported to the river through surface runoff in 2008 and, in contrast, were adsorbed into the dry ground in 2009.

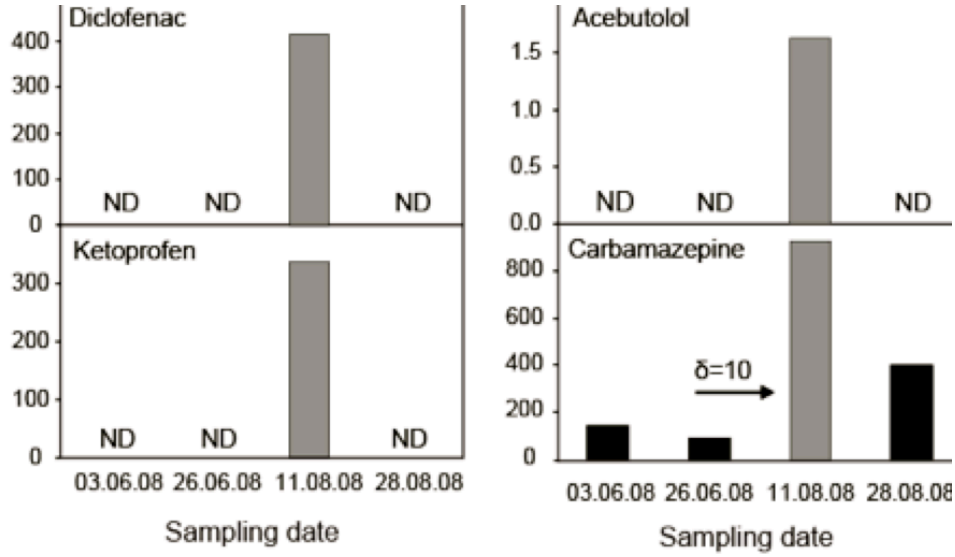


Figure 9. Mass flows of some of the monitored pharmaceuticals calculated from measurements done before, 24h after and well after the festival. A clear peak is observed in samples collected on August 11th 2008, i.e. 24h after the festival. Reprinted from "Neglected sources of pharmaceuticals – footprints of a reggae festival", Daneshvar et al., Journal of Environmental Monitoring, 14 (2012): 296-603.

Phototransformation of AIs (papers III-IV)

Diclofenac (Paper III)

The objective of this work was to investigate the aqueous photochemical transformation of diclofenac and its major transformation products (TPs) (8-chloro-9H-carbazol-1-yl) acetic acid (Cz1), 2-(2-chloro-phenylamino)-benzaldehyde (Ald) and the previously unreported (1,4-dioxo-4,9-dihydro-1H-carbazol-8-yl) acetic acid (Cz4).

Diclofenac is an anti-inflammatory drug that has been found in the aquatic environment on numerous occasions (e.g. Vieno 2007; Daneshvar 2009; Fick 2011; Scheurella 2009). Studies have also shown that the drug and its metabolites accumulate in fish, following

prolonged exposure to environmentally relevant concentrations (Schwaigera 2004; Kallio 2010). Phototransformation is regarded as its main route of transformation and over recent years several studies have been published on this subject (Moore 1990; Tixier 2003; Packer 2003). In-depth photokinetic studies on both the parent drug and its intermediate photoproducts are helpful in the elucidation of reaction scenarios that are likely to take place in the environment and during wastewater treatment.

All transformation experiments were performed in a previously constructed reaction chamber (Eriksson 2004), using a fluorescent tube with an emission spectrum similar to sunlight. A schematic illustration of the chamber is provided in Figure 10.

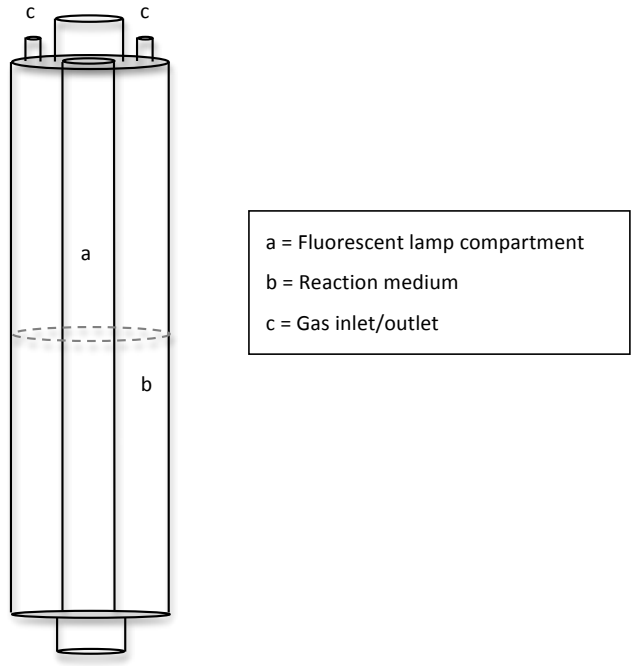


Figure 10. Schematic illustration of the reaction chamber used in the phototransformation experiments.

Upon irradiation of aqueous diclofenac it was concluded that the drug transforms by two major pathways (Figure 11).

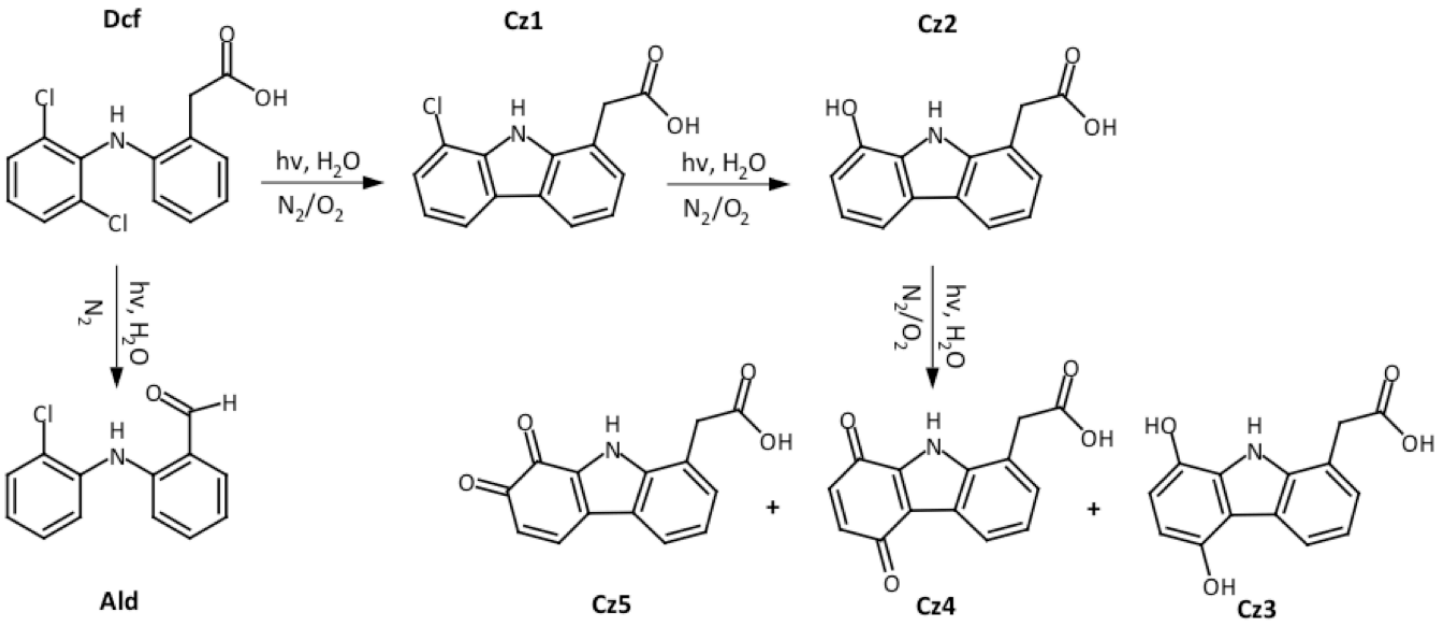


Figure 11. Phototransformation of aqueous diclofenac. Adapted from "A Photochemical Study of Diclofenac and its Major Transformation Products", Photochemistry and Photobiology, 86 (2010): 528-532.

The two primary products, Cz1 and Ald, were identified by comparison of retention times in LC- chromatograms with those of previously synthesized standards (paper V). Of these two, Ald has recently been identified as a TP with enhanced phytotoxicity (Schulze 2010). Cz1 was found to be the dominating direct photochemical reaction product of diclofenac, which is in agreement with earlier studies (Moore 1990). For unknown reasons Ald was only formed during deaerated conditions and despite exhibiting strong absorbance within the studied wavelength region (maximum at 385nm) Ald showed very low photochemical reactivity ($t_{1/2}$ = 3400 min). Further irradiation experiments conducted on Cz1 corroborated the suggested reaction pathway (Figure 11) as Cz4 was produced as the main end product via intermediate product Cz3. The absorption spectrum of Cz1 showed strong absorptions quite far into the sunlight wavelength region (λ_{max}= 324 and 337nm) and the photosensitive nature of the compound was further underlined by a rather high disappearance quantum yield (Φ = 0.09). After isolation and purification with semi-preparative HPLC, NMR-analyses confirmed the structure of Cz4. Since Cz4 was formed under both aerated and deaerated reaction conditions the two additional oxygen atoms found in its structure most probably originate from water. Quantification of the isolated product using H^1-NMR with 1,2-dichloroethane as internal standard also enabled calculation of its disappearance quantum yield.

Of the identified transformation products in this study the quinones (Cz3 and Cz4) have not previously been reported, even though the two isomers together accounted for approximately 50% of the starting amount of diclofenac following 200min of irradiation. Ald was the most stable transformation product but its importance may be questionable since the total amount formed during the experiments was less than 2% of the initial diclofenac concentration. Also, the presence of dissolved oxygen seems to hamper its formation.

Thyroxine (Paper IV)

Until recently, the occurrence of T_4 in the aquatic environment had not been determined and, consequently, there has been no obvious reason to investigate the photochemical behavior of the drug at environmentally relevant conditions. Previous photochemical work on T_4 has been limited to some synthetic applications, i.e preparation of isotopically labeled T_4-metabolites through photolysis of labeled thyroxine (van der Walt 1981).

As was concluded in paper I, T_4 is a surface water contaminant of potential concern and, accordingly, its transformation in the environment needs to be assessed. The aim of this work was to study the photochemical behavior of aqueous T_4.

The absorption spectrum of T_4 reveals that the compound exhibits high absorption in the relevant wavelength region with a maximum at 325nm and an extinction coefficient of 6 100 $M^{-1}cm^{-1}$ (pH 12). After irradiation of aqueous T_4 three different products or 'product categories' were identified (Figure 12). Of these compounds, P1 was structurally characterized with NMR after isolation and purification on HPLC, whereas P3 could be identified through the commercially available standard. Although the individual compounds constituting P2 were not fully characterized, plausible structures were assigned on the basis of HRMS and MS/MS analyses (Figure 12).

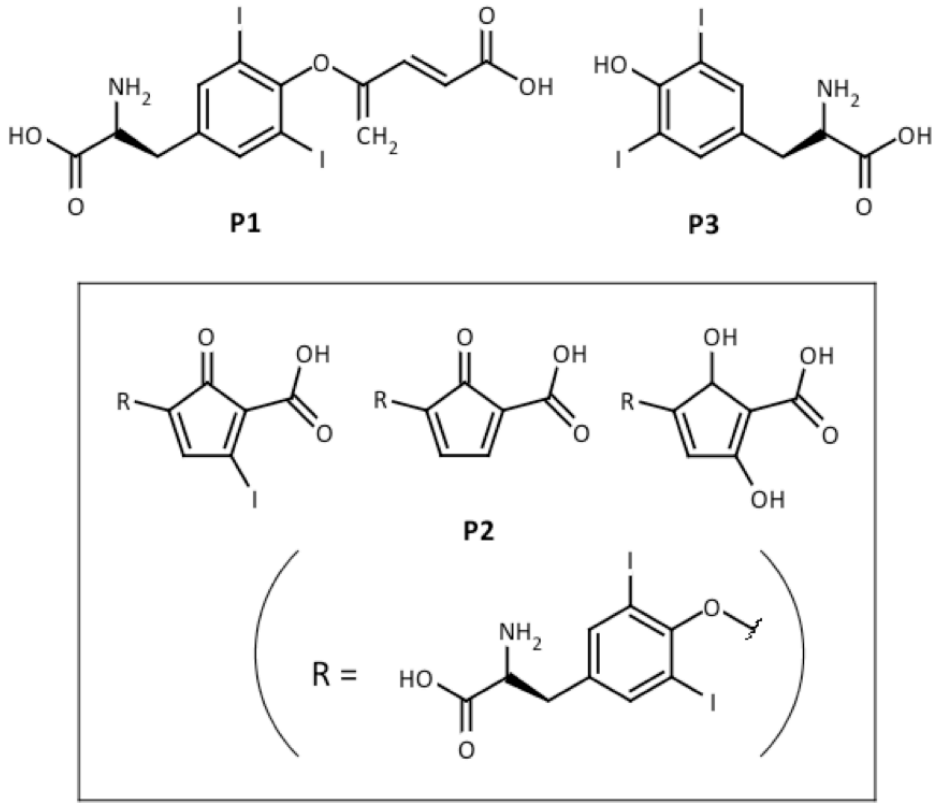


Figure 12. Chemical structures of T_4 PTPs. P1 and P3 were assigned by NMR and HRMS, while the P2 structures were tentatively assigned solely on the basis of MS data.

The results of the irradiation experiments indicate that the photoreactivity of T_4 resides on the phenolic subunit of the molecule. Three possible 'fates' of the phenolic moiety in T_4 were identified (Figure 12): 1) disintegration to an unsaturated aliphatic carboxylic acid (P1); 2) disintegration and rearrangement to a cyclopentadienoic acid or; 3) complete removal through cleavage of the C-O ether linkage, either in one or several intermediate reaction steps. As P1 was found to be stable at the studied conditions, in contrast to P3, the results suggest that the presence of a free phenolic hydroxyl group is important in terms of photoreactivity. Previous studies on direct phototransformation of 2-halophenols indicate that the phenolate anion is directly involved in the formation

of cyclopentadienic acids (Rayne 2009). Whereas irradiation of undissociated 2-halophenols provide a mixture of two products; phenol and cyclopentadienic acid, irradiation of the phenolate anion results exclusively in cyclopentadienic acid (Rayne 2009). The suggested mechanism is shown in Figure 13.

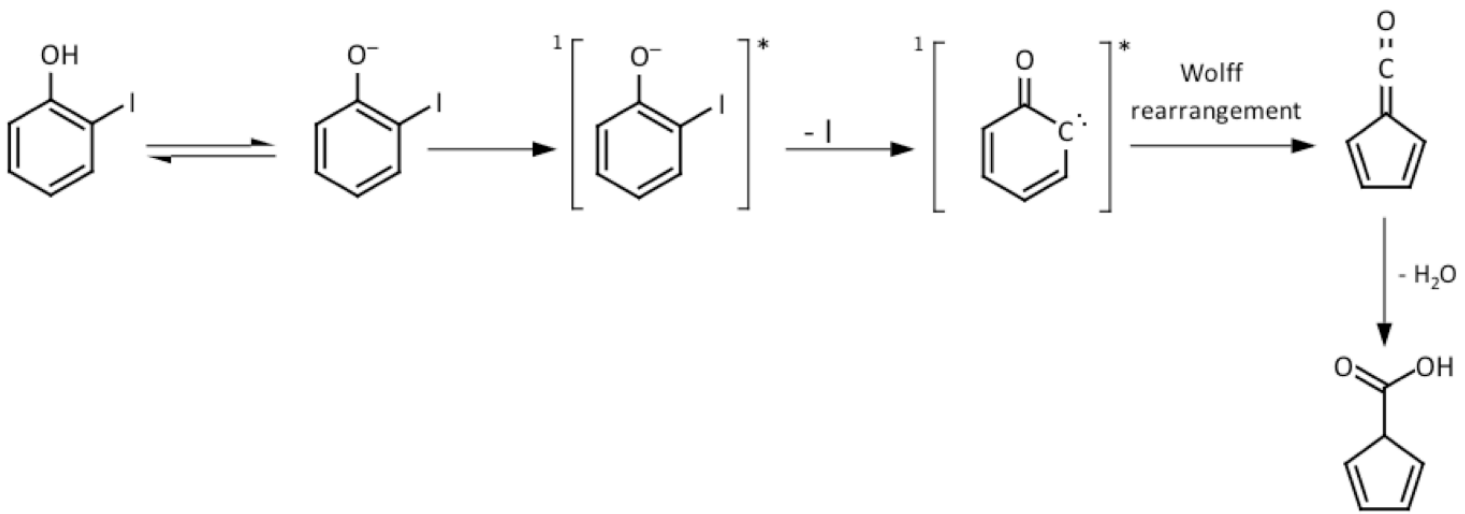


Figure 13. Mechanism for photochemical formation of cyclopentadienic acids from *ortho*-halogenated phenols. Adapted from ”Mechanistic aspects regarding the direct aqueous environmental photochemistry of phenol and its simple derivatives. A review.” by Rayne et al., Environment International, 35 (2009): 425-437.

Synthesis of phototransformation products (paper V)

Carbazoles

The intramolecular photocyclization of diclofenac to yield (8-chloro-9H-carbazol-1-yl) acetic acid (C-1) was originally identified as the main phototransformation route by Moore et al. in 1990 (Moore 1990). This reaction has been ascribed to the presence of the 2,6-dichlorodiphenylamine moiety, considered to be the photoactive chromophore of diclofenac (Encinas 1998). As described in paper V, both C-1 and 8-chloro-9H-carbazole-1-carboxylic acid (C-3) were synthesized photochemically by irradiating the compounds, dissolved in acetone, with UV-light. This synthetic approach was found to work well for C-3, as the compound was obtained in high yield (83%) following purification with HPLC. Instead, the cyclization reaction of diclofenac was not found to be equally successful, which may be due to the fact that C-1 is less stable than C-3 and will continue to react.

1-chlorocarbazole (C-4) was obtained according to a slightly modified literature procedure (Katritzky 1988), starting from carbazole. The first step involved protection of the secondary amine in carbazole with a pyrrolidinomethyl group through a Mannich reaction. The N-substituted carbazole was treated with n-butyllithium in diethylether to give a lithium-pyrrolidinomethyl-carbazole complex, which was then reacted with the chlorine-source, in this case hexachloroethane. Finally, removal of the pyrrolydinomethyl group afforded the *ortho*-monochlorinated carbazole in good yield (76%). It is possible to chlorinate directly on carbazole, i.e. without amine protection, although this would severely affect the reaction yield (Katritzky 1988). Thus the pyrrolidinomethyl group is key to the success of the reaction and actually serves two purposes at once: it inhibits the acidic amino hydrogen from reacting with the base and it ensures *ortho*-addition of the electrophile by creating a stable lithium-complex.

Reactions involving the formation of carbon-carbon bonds are vital for the preservation of life and have played a central role in organic synthesis throughout history. To date,

there have been as many as five Nobel prizes awarded for reactions creating new C-C bonds. The most recent prize was awarded in 2010 in recognition of the development of methods for palladium catalyzed cross-coupling reactions. For the synthesis of 1-chloro-8-methyl-9H-carbazole (C-2) a recently described method for intramolecular palladium(II)-catalyzed formation of carbazoles from diarylamines was utilized (Liégault 2008). The previously synthesized 2-chloro-N-(2-methylphenyl)aniline served as reactant and the reaction was performed in pivalic acid with catalytic amounts of palladium(II)acetate and potassium carbonate refluxed at 110°C. As revealed by GC-MS analyses the conversion progressed very slowly and was incomplete even after 58h, at which point the reaction was stopped. The *ortho*-relationship of the substituents on the aromatic rings may influence the reaction rate by hampering the rotation around the C–N–C bonds and thereby the diarylamine from adopting a suitable conformation. After purification by flash column chromatography and preparative thin layer chromatography the product could be obtained in decent yield (27.9%).

Diphenylamines

There are a number of efficient methods available for synthesis of various diphenylamines by nucleophilic aromatic substitution, most of which employ transition metals as catalysts. Copper was one of the first transition metals to be used in organic synthesis and its properties have been eagerly explored by organic chemists for many years. Despite this, there are still some loose ends concerning the mechanistic aspects of copper catalysis. The catalytic effect is highly dependent on correctly chosen reaction conditions, such as the choice of copper source, base, ligands, solvents and other additives, and even marginal adjustments can have a significant impact on reaction yields. Due to the toxic nature of some transition metals, including copper, environmental issues also need to be considered during development of new catalytic systems and a high reusability of the catalyst is therefore important. For these reasons, the development of systems applicable to a wide range of reactants is vastly time-consuming work.

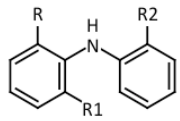
In this study, a synthetic route for C-N cross coupling of amines with iodobenzenes using copper(II)oxide nanopowder as catalyst was chosen for the synthesis of 2-chloro-N-phenylaniline (D-8), 2-chloro-N-(methylphenyl)aniline (D-4) and 2-(2-hydroxy-phenylamino)-benzoic acid (D-9) (Rout 2007). The catalytic activity of copper(II)oxide nanoparticles is suggested to reside on the surface of the particles and involve initial oxidative addition followed by a reductive elimination process (Rout 2007). Also, there are strong indications that the reactions are of heterogeneous nature (Rout 2007). Applied to he synthesis of D-4 and D-8, the method provided both products in decent yields (33.8% and 47.7%, respectively) after 24h of reaction. The reason for synthesizing D-9, which was not among the PTPs proposed by Agüera et al.(Agüera 2005), was that this compound could be considered a potential PTP of D-7 and, accordingly, could be used as a standard for further investigations of the phototransformation process. D-7 was one of the most abundant peaks in the LC-MS chromatogram presented in the paper by Agüera et al. (Agüera 2005). As expected, the reaction conditions were not optimal for the synthesis of D-9 and the pure product could only be obtained in low yield (7.5%). The two nucleophilic groups (–OH and $–NH_3$) in 2-aminophenol increased the risk of competing side-reactions as well as further reaction of the desired product. Again, the *ortho*-relationship of the substituents in the reactant molecules probably had an unfavorable effect on reaction yields.

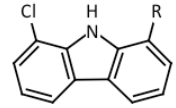
The compounds 2-[(2-chlorophenyl)amino]benzoic (D-7) acid and 2-[(2,6-dichlorophenyl)amino]benzoic acid (D-3) were synthesized in a commercial microwave using a procedure similar to the one described (Martín 2006). The reaction was performed in an open container under solvent-free conditions with coppper(II)sulphate as catalyst. At an operating power of 300W D-7 was obtained in good yield (69.1%) after only 4min. However, the reaction did not prove equally successful for the synthesis of D-8, as it could only be obtained in low yield (10.1%) following purification with HPLC. Steric hindrance as well as electronic deactivation, particularly prominent in the case of 2,6-dichloroaniline, probably affect the reactivity of the chloroanilines.

The alcohols, {2-[(2-chlorophenyl)amino]phenyl}methanol (D-5) and {2-[(2,6-dichlorophenyl)amino]phenyl}methanol, were conveniently synthesized by reduction of the corresponding carboxylic acid (previously prepared) with lithium aluminium hydride (LAH) in diethylether. Due to the poor ether-solubility of the carboxylic acids they were introduced to the reaction mixture in small portions using a Soxhlet extraction procedure. Both products were obtained in excellent yields, i.e. 91% and 81% for D-5 and D-2, respectively. According to Agüera et al. (Agüera 2005), one of the main PTPs formed during diclofenac phototransformation was 2-[(2-chlorophenyl)amino]benzaldehyde (D-6). This compound was subsequently prepared from D-5 by treatment with DDQ in dioxane for 30min. Separation by flash chromatography then provided the pure D-6 in good yield (63%). The decarboxylation product D-1 was prepared by boiling the sodium salt of diclofenac in N-methyl pyrrolidone. After clean-up, D-1 was obtained in 21% yield.

In conclusion, 13 potential PTPs of diclofenac were synthesized by utilizing a number of available synthetic methods (Table 5). It should be emphasized that the principal aim of the work was to obtain access to reference standards of PTPs and not to optimize individual reactions. Consequently, some of the reactions described still leave room for further improvements.

Table 5. Synthesized PTPs of diclofenac.





R	R1	R2	Yield (%)	Abbr.	R	Yield (%)	Abbr.
Cl	Cl	CH_2COOH	-	Diclofenac	CH_2COOH	17	C-1
Cl	Cl	CH_3	21	D-1	CH_3	27.9	C-2
Cl	Cl	CH_2OH	81	D-2	COOH	83	C-3
Cl	Cl	COOH	10.1	D-3	H	76	C-4
Cl	H	CH_3	33.8	D-4			
Cl	H	CH_2OH	91	D-5			
Cl	H	CHO	63	D-6			
Cl	H	COOH	69.1	D-7			
Cl	H	H	47.7	D-8			
OH	H	COOH	7.5	D-9			

Summary and conclusion

WWTPs are unquestionably the most important source of AIs in the aquatic environment and, as shown in this thesis, the thyroid hormone thyroxine can now be added to the growing list of AIs detected in WWTP effluents. The methodology applied to the analysis of thyroxine in an influent and effluent was described in detail in paper I. Apart from WWTPs, there are other, unexpected sources that, under the right circumstances, may become temporary hot spots of AIs. This was found to be the case for a reggae festival, arranged along the river Fyris in Uppsala, Sweden. The results of the analytical study presented in paper II concluded that runoff from the festival area, during a period of heavy rainfall, created mass flows of certain AIs that even exceeded the ones calculated from a nearby WWTP effluent.

After the environmental occurrence of a pollutant has been confirmed, a subsequent step usually involves assessment of its environmental fate. Phototransformation is considered an important transformation route for many AIs in the aquatic environment

and is thus frequently investigated. The aqueous phototransformations of diclofenac and thyroxine were studied in papers III and IV. It was concluded that both compounds give rise to several products upon exposure to UV-light. Some of the presented TPs, whose chemical structures were assigned during the study, have never previously been reported. The success of the photochemical work on diclofenac was made possible by the availability of reference standards of the major TPs, which had been synthetically prepared in a separate work. In conclusion, the results of the presented studies contribute with new and important knowledge concerning the photochemical behavior of AIs.

Finally, synthesis of some 13 potential PTPs of diclofenac was described (paper V). The main purpose of the work was to acquire access to major PTPs of diclofenac, in order to facilitate in-depth photochemical studies on the drug and to enable studies on the occurrence of the PTPs in the aquatic environment.

Bibliography

Agüera, A., Pérez Estrada, L., Ferrer, I., Thurman, E., Malato, S., Fernández-Alba, A. "Application of time-offlight mass spectrometry to the analysis of phototransformation products of diclofenac in water under natural sunlight." *Journal of Mass Spectrometry* 40 (2005): 908–915.

Barceló, D., Petrovic, M. "Challenges and achievements of LC-MS in environmentalanalysis: 25 years on." *Trends in Analytical Chemistry* 26 (2007): 2-11.

Bartels, P., von Tümpling Jr, W. "Solar radiation influence on the decomposition process of diclofenac in surface waters." *Science of the Total Environment* 374 (2007): 143–155.

Brozinski, J-M., Lahti, M., Oikari, A., Kronberg, L. "Detection of naproxen and its metabolites in fish bile following intraperitoneal and aqueous exposure." *Environmental Science and Pollution Research* 18 (2011): 811-818.

Canonica, S., Meunier, L., von Gunten, U. "Phototransformation of selected pharmaceuticals during UV treatment of drinking water." *Water Research* 42 (2008): 121-128.

Casson, L., Hunter, G., Bess Jr J. "The Challenge of the Future: Operating Emerging Disinfection Technologies." *Water Environment Federation's Annual Technical Exhibition and Conference 2006.* Dallas: Water Environment Foundation, 2006. 6361-6370.

Chiuminatto, U., Gosetti, F., Dossetto, P., Mazzucco, E., Zampieri, D., Robotti, E., Gennaro, M., Marengo, E. "Automated Online Solid Phase Extraction Ultra High Performance Liquid Chromatography Method Coupled with Tandem Mass Spectrometry for Determination of Forty-Two Therapeutic Drugs and Drugs of Abuse in Human Urine." *Analytical Chemistry* 82 (2010): 5636–5645.

Clara, M., Strenn, B., Kreuzinger, N. "Carbamazepine as a possible anthropogenic marker in the aquatic environment: investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration." *Water Research* 38 (2004): 947-954.

Daneshvar, A, Svanfelt, J., Kronberg, L., Weyhenmeyer, G. "Winter accumulation of acidic pharmaceuticals in a Swedish river." *Environmental Science and Pollution Research* 17 (2009): 908-916.

Daneshvar, A., Svanfelt, J., Kronberg, L., Prévost, M., Weyhenmeyer, G. "Seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in a Swedish river–lake system." *Chemosphere* 80 (2010): 301–309.

Daneshvar, A., Svanfelt, J., Kronberg, L., Weyhenmeyer, G. "Neglected sources of pharmaceuticals-footprints of a reggae festival." *Journal of Environmental monitoring* 14 (2012): 596-603.

Doerr-MacEwen, N.A., Haight M. E. "Expert Stakeholders' Views on the Management of Human Pharmaceuticals in the Environment." *Environmental Management* 38 (2006): 853–866.

Encinas, S., Bosca, F., Miranda, M. "Photochemistry of 2,6-D'ichlorodiphenylamine and 1-Chlorocarbazole, the Photoactive Chromophores of Diclofenac, Meclofenamic Acid and Their Major Photoproducts." *Photochemistry and Photobiology* 68 (1998): 640-645.

Eriksson, J., Rahm, S., Green, N, Bergman, Å., Jakobsson, E. "Photochemical transformation of tetrabromobisphenol A and related phenols in water." *Chemosphere* 54 (2004): 117-126.

Fick, J., Lindberg, R., Kaj, L., Brorström-Lundén, E. *Results from the Swedish National Screening Programme 2010 Subreport 3: Pharmaceuticals.* National study, Stockholm: Swedish Environmental Research Institute, 2011.

Flores-Valverde A., Horwood J., Hill E. "Disruption of the steroid metabolome in fish caused by exposure to the environmental estrogen 17alpha-ethinylestradiol." *Environemntal Science and Technology* 44 (2010): 3552-3558.

Garrison A. W., Pope, J. D. et al. "GC/MS analysis of organic compounds in domestic wastewaters." *Chem. Congr. North Am. Cont.*, 1975.

Garrison, A. W., Pope, J. D. "GC/MS analysis of organic compounds in domestic wastewaters." *Chem. Congr. North Am. Cont.*, 1975.

Gedye, R., Westaway, S., Ali, H., Baldisera, L., Laberge, L. Rousell, J. "The use of microwave ovens for rapid organic synthesis." *Tetrahedron Letters* 27 (1986): 279–282.

Giguere, R., Bray, T., Duncan, S., Majetich, G. "Application of commercial microwave ovens to organic synthesis." *Tetrahedron Letters* 27 (1986): 4945–4948.

Guardabassi L., Petersen A., Olsen J., Dalsgaard A. "Antibiotic resistance in Acinetobacter spp. isolated from sewers receiving waste effluent from a hospital and a pharmaceutical plant." *Applied and environmental biology* 64 (1998): 3499-3502.

Halling-Sørensen B., Nors Nielsen S., Lanzky P.F., Ingerslev F., Holten Lützhøft H.C., Jørgensen S.E. "Occurrence, fate and effects of pharmaceutical substances in the environment--a review." *Chemosphere* 36 (1998): 357-393.

Hignite, C. and Azarnoff, D. L. "Drugs and drug metabolites as environmental contaminants: chlorophenoxyisobutyrate and salicylic acid in sewage water effluent." *Life Sciences* 20 (1977): 337-341.

Jones, O., Voulvoulis, N., Lester J. "Human Pharmaceuticals in Wastewater Treatment Processes." *Critical Reviews in Environmental Science and Technology* 35 (2005): 401-427.

Kallio, J-M., Lahti, M., Oikari, A., Kronberg, L. "Metabolites of the Aquatic Pollutant Diclofenac in Fish Bile." *Environmental Science and Technology* 44 (2010): 7213–7219.

Kappe, C., Dallinger, D., Murphree, S. *Practical Microwave Synthesis for Organic Chemists: Strategies, Instruments, and Protocols.* Weinheim: WILEY-VCH Verlag GmbH & Co. , 2009.

Katritzky, A., Rewcastle, G., Vasquez de Miguel, L. "Improved syntheses of substituted carbazoles and benzocarbazoles via lithiation of the (dialkylamino)methyl (aminal) derivatives." *Journal of Organic Chemistry*, 1988: 794-799.

Khetan, S., Collins, T. "Human Pharmaceuticals in the Aquatic Environment: A Challenge to Green Chemistry." *Chemical Reviews* 107 (2007): 2319-2364.

Kim S., Aga D. "Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants." *ournal of toxicology and environmental health. Part B, critical reviews.* 10 (2007): 559-573.

Kolpin, D., Furlong, E., Meyer, M., Thurman, E., Zaugg, S., Barber, L., Buxton, H.,. "Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. strems, 1999-2002: A national reconnaissance." *Environmental Science and Technology* 36 (2002): 1202-1211.

Kosonen, J., Kronberg, L. "The occurrence of antihistamines in sewage waters and in recipient rivers." *Environmental Science and Pollution Research* 16 (2009): 555-564.

Kostich, MS., Lazorchak, JM. "Risks to aquatic organisms posed by human pharmaceutical use." *Science of the Total Environment* 389 (2008): 329-339.

Liégault, B., Lee, D., Huestis, M., Stuart, D., Fagnou, K. "Intramolecular Pd(II)-catalyzed oxidative biaryl synthesis under air: reaction development and scope." *Journal of Organic Chemistry* 73 (2008): 5022–5028.

Lindström, P., Tierney, J., Wathey, B., Westman, J. "Microwave assisted organic synthesis - a review." *Tetrahedron* 57 (2001): 9225-9283.

Martín A., Mesa M., Docampo M., Gómez V., Pellón R. "Fast synthesis of substituted N-phenylanthranilic acids using Ullmann condensation under microwave irradiation in dry media." *Synthetic Communications* 36 (2006): 271–277.

Martínez Bueno, M., Agüera, A., Hernando, M., Gómez, M., Fernández-Alba, A. "Evaluation of various liquid chromatography-quadrupole-linear ion trap-mass

spectrometry operation modes applied to the analysis of organic pollutants in wastewaters." *Journal of Chromatography A* 1216 (2009): 5995–6002.

Mauzerall, D. "The photochemical origins of life and photoreaction of ferrous ion in the archaean oceans." *Origins of Life and Evolution of Biospheres* 20 (1990): 293-302.

McGlade, J. *EEA Technical report no 1/2010. Pharmaceuticals in the environment - Results of an EEA workshop.* Technical report, European Environment Agency, Copenhagen: European Environment Agency, 2010.

Melouna, M., Bordovská, S., Galla, L. "The thermodynamic dissociation constants of four non-steroidal anti-inflammatory drugs by the least-squares nonlinear regression of multiwavelength spectrophotometric pH-titration data." *Journal of Pharmaceutical and Biomedical Analysis* 45 (2007): 552–564.

Moore, D., Roberts-Thomson, S., Zhen, D., Duke, C. "Photochemical studies on the antiinflammatory drug diclofenac." *Photochemistry and Photobiology* 52 (1990): 685-690.

Norwegian Institute for Water Research (NIVA). *ER09: Levels of Pharmaceuticals and Drugs of Abuse in the Scottish Environment.* Scotland and Northern Ireland Forum for Environmental Research, 2009.

Packer, J., Werner, J., Latch, D., McNeill, K., Arnold, W. "Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen." *Aquatic Sciences* 65 (2003): 342-351.

Petrovic, M., Barceló, D. "LC-MS for identifying photodegradation products of pharmaceuticals in the environment." *Trends in Analytical Chemistry* 26 (2007): 486–493.

Pilkington, N. "Disinfection of water and waste water by ultra violet irradiation." In *Modern techniques in water and wastewater treatment*, by L., Priestley, A. Kolarik. Melbourne: CSIRO Publishing, 1995.

Poiger, T., Buser, H-R., Müller, M. "Photodegradation of the pharmaceutical drug diclofenac in a lake: Pathway, field measurements, and mathematical modeling." *Environmental Toxicology and Chemistry* 20 (2001): 256–263.

Radke, M., Ulrich, H., Wurm, C., Kunkel, U. "Dynamics and Attenuation of Acidic Pharmaceuticals along a River Stretch." *Environmental Science and Technology* 44 (2010): 2968–2974.

Rayne, S., Forest, K., Friesen, K. "Mechanistic aspects regarding the direct aqueous environmental photochemistry of phenol and its simple halogenated derivatives. A review." *Environment International* 35 (2009): 425–437.

Rout, L., Jammi, S., Punniyamurthy, T. "Novel CuO Nanoparticle catalyzed C-N cross coupling of amines with iodobenzene." *Organic Letters* 9 (2007): 3397–3399.

Sadezky, A., Löffler, D., Schlüsener, Roig, B., Ternes, T. "Chapter 4. Real situation: Occurrence of the main investigated PPs in water bodies." In *Pharmaceuticals in the environment - current knowledge and need assessment to reduce presence and impact*, by Benoit Roig, 31-. London: IWA Publishing, 2010.

Scheurella, M., Frankea, S., Shahb, R., Hühnerfuss, H. "Occurrence of diclofenac and its metabolites in surface water and effluent samples from Karachi, Pakistan." *Chemosphere* 77 (2009): 870-876.

Scheytt, T., Mersmann, P., Lindstädt, R., Heberer, T. "1-Octanol/Water Partition Coefficients of 5 Pharmaceuticals from Human Medical Care: Carbamazepine, Clofibric Acid, Diclofenac, Ibuprofen, and Propyphenazone." *Water and Air Pollution* 165 (2005): 3-11.

Schulze, T., Weiss, S., Schymanski, E., Carsten von der Ohe, P., Schmitt-Jansen, M., Altenburger, R., Streck, G., Brack, W. "Identification of a phytotoxic photo-transformation product of diclofenac using effect-directed analysis." *Environmental Pollution* 158 (2010): 1461-1466.

Schwaigera, J., Ferlinga, H., Mallowa, U., Wintermayrb, H., Negele, R. "Toxic effects of the non-steroidal anti-inflammatory drug diclofenac ☆: Part I: histopathological alterations and bioaccumulation in rainbow trout." *Aquatic Toxicology* 68 (2004): 141-150.

Stumm-Zollinger, E., Fair G. M. "Biodegradation of steroid hormones." *Journal of the Water Pollution Control Federation* 37 (1965): 1506-1510.

Svanfelt, J., Eriksson, J., Kronberg, L. "A Photochemical Study of Diclofenac and Its Major Transformation Products." *Photochemistry and Photobiology* 86 (2010): 528–532.

Svanfelt, J., Eriksson, J., Kronberg, L. "Analysis of thyroid hormones in raw and treated waste water." *Journal of Chromatography A* 1217 (2010): 6469–6474.

Svanfelt, J., Kronberg, L. "Synthesis of substituted diphenylamines and carbazoles: phototransformation products of diclofenac." *Environmental Chemistry Letters* 9 (2011): 141-144.

Tabak, H. H. and Bunch, R. L. "Steroid hormones as water pollutants. I. Metabolism of natural and synthetic ovulation-inhibiting hormones by microorganisms of activated sludge and primary settled sewage." *Dev. Ind. Microbiol.* 11 (1970): 367-376.

Tchobanoglous, G. Burton, F., Stensel, D. *Wastewater Engineering Treatment and Reuse.* 4th Edition. New York: McGraw Hill Higher Education , 2002.

Ternes, T. "Occurrence of drugs in German sewage treatment plants and rivers." *Water Research* 32 (1998): 3245-3260.

Tixier, C., Singer, H., Oellers, S., Müller, S. "Occurrence and Fate of Carbamazepine, Clofibric Acid, Diclofenac, Ibuprofen, Ketoprofen, and Naproxen in Surface Waters." *Environmental Science and Technology* 37 (2003): 1061–1068.

Trenholm R., Vanderford B., Snyder S. "On-line solid phase extraction LC-MS/MS analysis of pharmaceutical indicators in water: a green alternative to conventional methods." *Talanta* 79 (2009): 1425-1432.

van der Walt, B., Cahnmann, H. "Synthesis of thyroid hormone metabolites by photolysis of thyroxine and thyroxine analogs in the near UV." *Proceedings of the National Academy of Sciences of the United States of America* 79 (1981): 1492-1496.

Vieno, N. *Occurrence of pharmaceuticals in Finnish sewage treatment plants, surface waters and their elimination in drinking water treatment processes.* PhD thesis, Tampere University of Technology, Tampere: Tampere University of Technology, 2007.

Viglino, L., Aboulfadl, K., Daneshvar Mahvelat, A., Prévost, M., Sauvé, S. "On-line solid phase extraction and liquid chromatography/tandem mass spectrometry to quantify pharmaceuticals, pesticides and some metabolites in wastewaters, drinking, and surface waters." *Journal of environmental monitoring* 10 (2008): 482-489.

Viglino, L., Aboulfadl, K., Prévost, M., Sauvé, S. *Detection of Pharmaceuticals, Personal Care Products, and Pesticides in Water Resources by APCI-LC-MS/MS.* Application note: 466, San Jose: Thermo Fisher Scientific Inc., 2009.

Wardle, B. *Principles and Applications of Photochemistry.* Chichester, West Sussex: John Wiley & Sons, Ltd, 2009.

Waters Corporation. *Environmental and Agrochemical Applications Notebook.* Catalogue, Massachussetts: Waters Corporation, 2002.

Won, C. M. "Kinetics of degradation of levothyroxine in aqueous solution and in solid state." *Pharmaceutical Research* 9 (1992): 131-137.

Zhang Y., Marrs C., Simon C., Xi C. "Wastewater treatment contributes to selective increase of antibiotic resistance among Acinetobacter spp." *Science of the total environment* 407 (2009): 3702-3706.

Zhang, F., Bartels, M., Brodeur, J., McClymont, E. Woodburn, K. "Quantitation of 17α-ethinylestradiol in aquatic samples using liquid-liquid phase extraction, dansyl derivatization, and liquid chromatography/positive electrospray tandem mass spectrometry." *Rapid Communications in Mass Spectrometry* 18 (2004): 2739-2742.

Zhou, H. Smith, D. "Advanced technologies in water and wastewater treatment." *Canadian Journal of Civil Engineering* 28 (2001): 49-66.

Jesper Svanfelt

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