Textile Related Chemicals: Analytical Approaches Towards the Assessment of Human and Environmental Exposures

Francesco Iadaresta
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Abstract
The textile manufacturing chain involves an extensive use of chemicals as early as fabric-production. To confer special features to textile materials, more chemicals are required in subsequent steps. Furthermore, potentially harmful substances can end up in clothes as transformation products. Compounds that are not covalently bonded to the fabrics have high probability to be released on the skin or into the environment when the clothes are worn or laundered.

In order to remove interfering compounds from solvent extracts of investigated textiles, a cleanup step based on solid phase extraction using graphitic carbon black was developed resulting in effective dye removal. In a pilot screening, nitroanilines were detected up to 0.57 mg/g, which was 2-3 order of magnitude higher than measured quinolines.

Human exposure to chemicals can occur through skin contact. Benzo[b]thiazole was chosen as model compound for in-vitro experiments. Its permeation was experimentally determined in order to estimate dermal exposure. Carcinogenic and non-carcinogenic risks, associated to wearing t-shirt containing BT, according to international standards, were found to be below the acceptable exposure levels.

It has been shown that chemical concentrations decreased during domestic washing. A procedure was developed for enrichment and clean-up of textile related compounds from water samples. The method was applied to three wastewater treatment plant effluents located in Stockholm. Tolyltriazole, 1-benzotriazole, and UV-P were detected within the range of 53-1148 ng/L.

Suspect and non-target screening methodology was developed do detect and identify substances in textile materials. The occurrence of thirteen suspect compounds, belonging to quinolines, nitroanilines, benzo[b]thiazoles, benzo[b]thiazoles and phthalates, was confirmed through suspect analysis approach. Furthermore, using a non-target screening approach, compounds not included in the suspect list such as nitrophenols, organophosphate and acridine were identified.

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Keywords: graphitized carbon black- GCB, franz cell, diffusion cell, risk assessment, screening, textile.
TEXTILE RELATED CHEMICALS: ANALYTICAL APPROACHES TOWARDS THE ASSESSMENT OF HUMAN AND ENVIRONMENTAL EXPOSURES

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Francesco Iadaresta
To my beloved Family
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Abstract

The textile manufacturing chain involves an extensive use of chemicals as early as fabric-production. To confer special features to textile materials, more chemicals are required in subsequent steps. Furthermore, potentially harmful substances can end up in clothes as transformation products. Compounds that are not covalently bonded to the fabrics have high probability to be released on the skin or into the environment when the clothes are worn or laundered.

In order to remove interfering compounds from solvent extracts of investigated textiles, a cleanup step based on solid phase extraction using graphitic carbon black was developed resulting in effective dye removal. In a pilot screening, nitroanilines were detected up to 0.57 mg/g, which was 2-3 order of magnitude higher than measured quinolines.

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Suspect and non-target screening methodology was developed to detect and identify substances in textile materials. The occurrence of thirteen suspect compounds, belonging to quinolines, nitroanilines, benzotriazoles, benzothiazoles and phthalates, was confirmed through suspect analysis approach. Furthermore, using a non-target screening approach, compounds not included in the suspect list such as nitrophenols, organophosphate and acridine were identified.
Populärvetenskaplig sammanfattning

Produktionen av textiler är en global, komplex process där ett stort antal olika kemikalier används i de olika tillverkningsstegen. Några av de kemikalier vi i denna studie detekterat i kläder som säljs på marknaden har hälsoskadliga effekter, till exempel genom att vara cancerframkallande eller orsaka hudallergi. På grund av det stora antal olika kemikalier i klädtillverkningen, och att det idag finns få regleringar angående vilka ämnen som får finnas i kläder som säljs till konsument, krävs det att man utvecklar analytiska metoder för att identifiera föreningar med potentiella hälsoskadliga effekter i kläder. I denna avhandling beskrivs analytiska strategier och metoder för bestämning av kemikalier som kan finnas i kläder sålda på marknaden.

Avhandlingen fokuserar på små molekyler som inte är kemiskt bundna till textilerna. Dessa substanser har hög sannolikhet att överföras från textilmaterialet till huden genom den dagliga användningen av kläderna. En annan möjlig väg för dessa kemikalier är att de kan transporteras till miljön genom tvättning av kläder, och därigenom kan textilier vara en potentiell källa till miljöföroreningar.

Avhandlingen undersöker även hudexponeringen av bensotiasol. Det valdes som modellsubstans då det har detekterats i en stor del av de kläder som har undersökts. Via dessa *in vitro*-studier gjordes en uppskattning av humanexponering för bensotiasol i textilmaterialet. Studien visade på att exponering av bensotiasol genom daglig användning av kläder ligger lägre än en acceptabel risknivå.

Tidigare studier har visat att mängden kemikalier i kläder minskat vid tvättning. I denna avhandling beskrivs metoder för att mäta vanligt
förekommande textilrelaterade kemikalier i utgående vatten från vattenreningsverk. Avloppsvatten är en komplex matris, som innehåller många olika föreningar i relativt låga halter, och det krävs därför en effektiv och selektiv upprening av de specifika föreningar som man vill detektera. I avhandlingen presenteras analytiska metoder för haltbestämning av ett antal av textilkemikalierna i vatten.
List of Papers

The thesis is a compilation of four scientific papers focus on the different aspects of chemicals in textiles, which will be denoted with their Roman numerals.


   *The author was responsible for ideas and planning, part of experimental work, significant part of writing and significant data analysis. (First author shared)*


   *The author was responsible for ideas and planning, involved in part of experimental work, major part of writing and data processing.*


   *The author was responsible for ideas and planning, involved in all experimental work, major part of writing and data processing.*


   *The author was responsible for planning, for part of experimental work, major part of writing and data analysis.*
List of publications not included in the thesis


## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>BFR</td>
<td>Bundesinstitut für Risikobewertung</td>
</tr>
<tr>
<td>BT</td>
<td>Benzothiazole</td>
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<tr>
<td>C.I.</td>
<td>Color index</td>
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<tr>
<td>CLP</td>
<td>Classification, Labeling and Packaging</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
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<tr>
<td>C-trap</td>
<td>Curved linear trap</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DDA</td>
<td>Data Dependent Acquisition</td>
</tr>
<tr>
<td>DIA</td>
<td>Data Independent Acquisition</td>
</tr>
<tr>
<td>DMD-HEU</td>
<td>Dimethylol dihydroxy ethylene urea</td>
</tr>
<tr>
<td>GCBs</td>
<td>Graphitized Carbon Black</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HQ</td>
<td>Hazard quotient</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass over charge ratio</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>PBDEs</td>
<td>Polybrominate diphenyl ethers</td>
</tr>
<tr>
<td>PCBs</td>
<td>Polychlorinated biphenyls</td>
</tr>
<tr>
<td>PET</td>
<td>Poly-(ethylene terephthalates)</td>
</tr>
<tr>
<td>PGC</td>
<td>Porous graphitic carbon</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>PREG</td>
<td>Polar retention effect on graphite</td>
</tr>
<tr>
<td>PRM</td>
<td>Parallel Reaction Monitoring</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinyl chloride</td>
</tr>
<tr>
<td>QIT</td>
<td>Linear ion trap mass analyzer</td>
</tr>
<tr>
<td>QqQ</td>
<td>Triple quadrupole mass analyzer</td>
</tr>
<tr>
<td>QuEChERS</td>
<td>Quick, easy, cheap, effective, rugged and safe</td>
</tr>
<tr>
<td>REACH</td>
<td>Registration, Evaluation, Authorization and Restriction of Chemicals</td>
</tr>
<tr>
<td>RfD</td>
<td>Reference dose</td>
</tr>
<tr>
<td>SIM</td>
<td>Selected ion monitoring</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>SRM</td>
<td>Selected Ion Monitoring</td>
</tr>
<tr>
<td>SVHC</td>
<td>Substances of Very High Concern</td>
</tr>
<tr>
<td>WWTP</td>
<td>Wastewater treatment plant</td>
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1 Introduction

In this thesis the presence of chemicals in clothing textile materials is investigated, using different analytical methods to evaluate related possibilities for human exposure and environmental pollution.

1.1 Textiles

Textile, from the Latin textilis, meaning “woven”, originally was used to refer to intertwined fabrics. Nowadays, the term includes a large variety of natural or synthetic materials produced through different methods (e.g., knitting, felting, bonding, etc.) and made from numerous types of filaments [1]. Initially, the main use of textile was protection, however, there are evidence of dyed fabrics back to prehistoric times, showing human interest in other functions such as aesthetics and comfort [2]. Textile production in our time, involve an extensive use of chemicals in the whole production chain, which will be discussed in the following sections.

1.2 Textile production processes

The textile manufacturing chain begins with the production of raw fibers, which can be categorized as natural and synthetic. Textile fibers are large polymeric molecules composed of repeating subunits belonging to five major chemical types [3]:

- protein (e.g. wool),
- polyamide (e.g., nylon),
- polyester (e.g. polyethylene terephthalate),
1.2.1 Natural fibers

The textile industry is one of the world’s largest water user, since a vast amount of water is required throughout the entire manufacturing process, starting from the cultivation of natural fibers [4].

Among natural fibers, cotton is the most important, accounting for 30% of the world fiber consumption [5]. Cotton is a cellulosic natural fiber obtained from renewable sources and the production accounts for 2.6% of the overall global water use. Water footprint is a measure of water use in the production of goods [6]. Generally, the impact of water use in the production is not included in the price of the final product, with the exception of the cost made for wastewater treatment [6]. However, only few industrialized countries reach the level of 100% wastewater treatment, while for developing countries this percentage often remains below 5% [7]. In most of the cases the percentage of treated water is related only to household and industrial water flows, and does not consider the diffuse waste water flow from agricultural use [6]. In addition to a large water consumption, traditional cultivation of cotton requires a heavy use of pesticides; it is globally estimated that only 3% of cultivated lands are devoted to cotton production, whereas they consume 25% of globally used pesticides [8,9]. Moreover, herbicides (defoliants) are used before the cotton harvest, and residues of these can be found on the final fiber [10].

The most significant animal fiber used in textiles is sheep wool [9], which also is a renewable resource. In contrast to vegetable fibers, wool does not require herbicides or pesticides for the production, whilst parasiticides are frequently applied on the sheep coat [11]. Wool production can have a
negative impact to the environment in another way, e.g. generating soil erosion through sheep overgrazing or creating runoff contamination by animal manure [9].

1.2.2 Synthetic fibers

Synthetic fibers accounted for 62% of total worldwide fiber consumption in 2018 [5]. Among the many kinds of synthetic fibers, nylon and polyester are the most common. These are produced from non-renewable petroleum resources and are essentially non-biodegradable [9].

Nylon, which was the first produced synthetic fiber [12], is a polymer based on aliphatic or semi-aromatic polyamides. The many nylon types differ by the number of carbon atoms in the respective monomeric units. Nylon 6,6 and nylon 6 are by far the most important commercial polyamide polymers. The former is composed by diamine and di-acidic subunits having six carbon atoms each, while the latter is composed of a single subunit consisting of a 6 carbon atom caprolactam-derived amino-acid [13], structures are showed in Figure 1.

![Chemical structure of units for Nylon 6,6 (a) and Nylon 6 (b)](image)

**Figure 1**: Chemical structure of units for Nylon 6,6 (a) and Nylon 6 (b)

Poly-(ethylene terephthalates) or PET is the largest volume produced synthetic fiber, with 21 million metric tons produced in 2002 alone, Figure 2. It has been estimated that with this amount it would be possible to make a filament (≈20μm in diameter) stretching to 0.01 light years (≈9.4*10^12 km), corresponding to one million trips to the moon [13].

13
Environmental impact of nylon and PET production can be related to air pollution (e.g., emission of carbon oxides), water pollution (release of toxic agents, chemicals and petroleum run-off), land pollution (discharge of chemicals toxic to animals and/or plants), and noise pollution [14]. An assessment of environmental impacts associated to fabric production was estimated for hotel textiles made in cotton and polyester [15]. Cotton fiber production was estimated to consume 40% less energy than polyester fiber production. However, the study mentioned the high use of pesticides and water consumption in cotton production. It was also concluded that blended textile (cotton and polyester, 50:50) resulted in less environmental impact since these fabrics have longer durability as well as lower laundering energy requirement.

1.2.3 Finishing processes in textile production

Regardless of natural or synthetic origin, there is a wide use of chemicals involved already in the production of textile fibers [9]. The textile industry in turn, applies extensive use of chemicals in subsequent processes to manufacture the textile materials, e.g. pretreatment, dyeing, printing, finishing, and coating, as well as washing and drying [16]. Due to the large number of steps involved in the finishing processes, only some of them
will be discussed, providing some examples to obtain an overview of the chemicals involved.

1.2.3.1 Fabric pretreatments

Each kind of fiber requires an appropriate pretreatment process to ensure material uniformity, removal of foreign matter, and increased dyestuff affinity.

A specific pretreatment is required for cotton fibers, which can proceed in further production steps only if the waxy outer layer is dissolved in aqueous sodium hydroxide. This treatment is called “scouring” and is required in order to allow dye penetration into the fibers [9].

Size materials (fillers) are used to increase fabric resistance and durability, and their excesses are successively removed in desizing processes. The washing water produced in the following desizing step contributes up to 70% of the chemical oxygen demand (COD) in textile industrial effluents [16]. The COD, expressed in milligrams of oxygen per liters consumed by a reaction in a solution, is used as indicator of water quality and it is the most widely used parameter to characterize wastewaters [17]. Chemicals released from this process, especially compounds difficult to emulsify or that hardly biodegrade such as silicone oils, can represent an environmental concern [16,18].

Fibers have their own color, stains, and impurities which are removed in a chemical process called bleaching, which depends on the kind of fiber. Since its introduction, hydrogen peroxide remains the most prominent bleaching agent [19]. The whiteness is obtained by an oxidation reaction which reduce the number of chromophore groups with highly conjugated systems [20]. The reaction is conducted at high temperature and alkaline conditions with the use of many auxiliary compounds such as stabilizers.
[21], activators and anticorrosion agents [22]. Stabilizers prevent pH changes or making complex with Iron (II) and Magnesium (II) which promote the catalysis of hydrogen peroxide decomposition [20].

1.2.3.2 Dyeing

Dyeing is the process that gives a specific color to textile materials. Color index (C.I.) is the most common dye classification, organizing dyes by their color and classifying them according to application methods and chemical structures [23]. Each C.I. name describes all the colorants having identical structure. However, this does not necessarily describes the very same product in terms of additives or impurity content. Furthermore, each manufacturer can invent a trade name for a given dye. Consequently, there are more than fifty thousand commercial names for dyes and several of them refer to the same product [24]. This generates confusion for the consumers and is making identification of the used dye difficult.

Dyes can be applied in different production steps and the process can be performed alone or at the same time with other treatments. The method of application depends on the fabric and the process can require up to 150 liters of water per kilogram of fabric [25]. Initially, dyes were designed to chemically react with functional groups of the natural fibers (e.g., –NH₂, –COOH, and –OH as in cotton and silk) and later synthetic fiber such as nylon. PET polymers, on the other hand, have a low affinity for traditional dye application methods due to the absence of available functional groups in their chemical structure. Instead, polyesters and all the other synthetic fabrics have the dyes applied in solution stage using a carrier or a combination of high temperature and pressure, before the formation of the fabric [9,13].

Dyes are classified according to how they are applied onto fabrics [24,26]:
- **Reactive dyes**: where the chromophore is directly bound to the fabric with a covalent bond. These are usually applied to cotton but also to wool and nylon [27].

- **Disperse dyes**: These are used for hydrophobic fibers, predominantly polyester. The increased use of this technique is closely related to the increasing use of polyester. It is also used for the coloration of nylon. These dyes are not soluble in water and need to be dispersed with the use of surfactants in dyebaths [28].

- **Direct dyes**: Dyes that are directly applied to cellulosic fibers from a hot aqueous solution. After the application a treatment that includes chelation with salts of heavy metals (copper or chromium) is required to improve the wash proof properties.

- **Vat dyes**: Soluble dyes in a water container (or vat), are transferred to the fabric, and then converted to insoluble dyes. An example of this is the application of indigo [29]. Indigo dyes are not soluble in water. However, if these are reduced to their soluble leuco form, it can penetrate the cotton fabrics. Subsequently, these are reoxidized to their insoluble keto form.

- **Sulfur dyes**: Used in cotton dyeing technique. These dyes are applied from an alkaline reducing bath containing sodium sulfide as reducing agent, in which the disulfide groups of the dyes are reduced to mercapto groups, then successive oxidation is performed after rinsing. These involve low cost and good wash proof properties, but present an environmental concern due to the high polluting nature of dye-bath waste water [24].

- **Acidic dyes**: Water soluble anionic dyes, applied to synthetic (nylon and modified acrylics) and natural fibers (wool and silk). These dyes enclose in their structures sulfonic or carboxylic salt
groups and are dyed in acidic solutions. In acidic conditions positively charged fabrics are responsible for dye diffusion and migration into the fabrics [30].

- Basic dyes (cationic): Water soluble cationic dyes, originally used for natural fibers such as silk and wool, find also applications in nylon and modified polyester dyeing. In analogy with acidic fabric they will be attracted by negative charge on the fabric during the application.

- Solvent dyes: Not water-soluble dyes which are soluble in organic solvent, having their main application for coloring plastics, oil, and waxes.

1.2.3.3 Other finishing treatments

Several other chemicals are used to improve specific properties of the textile fibers, such as appearance and/or performances. Textile materials are widely treated with formaldehyde releasing compounds and resins, to improve anti-wrinkling properties. For example, cellulose fibers are cross-linked with dimethylol dihydroxy ethylene urea (DMD-HEU). Its ether groups can gradually be hydrolyzed to N-methylol group, during the washing cycles [31], which exists in equilibrium with formaldehyde [32], and it is classified as carcinogenic to humans and also has contact allergic properties [33].

Phthalates are used to increase flexibility and durability of polyvinylchloride (PVC)-based textile coating used to generate patterns and prints on textiles [34]. Several of these chemicals have been related to reproductive toxicity.

Textiles, are also treated with flame retardants to inhibit ignition. Polybrominated diphenyl ethers (PBDEs) are used as flame retardants, but
are persistent environmental pollutants [35]. Furthermore, surfactants, such as nonylphenol ethoxylates, and biocides, for instance triclosan [36], are widely used in textile materials. The former are toxic to aquatic life, while the latter has been associated to endocrine disruption [37,38].

1.3 Chemicals in commercial clothing

After production, textile end-products enter the market: the annual consumption of textiles in Europe for 2012 was about 19 kg per citizen, of which two-thirds were clothes [39]. Chemicals that have been detected in finished textiles available on the open market can be divided in [40]:

1) Functional chemicals: added during the production to give certain properties to the textile and are meant to remain in the final article. Example of these are dyes, plasticizer and flame retardants.

2) Process chemicals (or auxiliary): used in the manufacturing processes, but not provide any special features to the final fabrics, and are not meant to remain in the textile material. Examples are biocides added in storage and/or transport, surfactants used as wetting agent in dyeing applications, or in the washing of the textile in between the different processes.

3) Unwanted chemicals: these do not have any function neither in the production nor in the final garment, but end up in the final product for example due to contamination or degradation reactions. Example of these are degradation products of the dyes, formaldehyde released from resins, metals (e.g. heavy metals), etc.

Although numerous chemicals and chemical treatments are used in the textile production, there is no comprehensive overview of the hazardous
substances actually occurring in clothing textiles. A summary have been made in a recent published review [41] and in a report from Bundesinstitut für Risikobewertung (BFR) [42].

1.3.1 Regulations

Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) and Classification, Labeling and Packaging (CLP) regulations are both important pieces of chemical legislation in Europe [43,44].

The large number of intermediaries and the rapid changes in the textile production chain, due for example to new fashion trends, complicates the registration process [45]. Moreover, manufacturers and importers are not obliged to register substances less than one ton per year. For this reason many imported chemicals that are below regulated amounts are not registered [40].

Although the Annex XVII of Regulation (EC) 1907/2006 contains restrictions for substances that are not allowed to be manufactured or used in Europe, the REACH regulation is not specified for chemicals in textile materials [46]. However, REACH bans the use of azo-dyes which can release twenty-two carcinogenic amines listed in Appendix 8, in amounts higher than 30 ppm. Recently, clothing, other textiles and footwear are considered a priority case in the amended EU regulation 2018/1513. This regulation further restricts the amounts of certain substances classified as carcinogenic, mutagenic or toxic for reproduction [47].

European regulation for eco-labeling restricts, among other chemicals used in textile production, some dispersive dyes that are listed as carcinogenic or allergenic [48].
1.4 Chemical release from textile materials

Chemicals used in textile production, together with possible post-production contaminations and the limitations of current regulations, make research on possible release of hazardous compounds contained in textile materials important. The release of chemicals from the textiles depends on intrinsic factors, such as type of fiber, dyeing process, and external factors like washing, drying and storage [40,49]. Substances can be released during wearing and/or laundering; the former related to human exposure, while the latter is related to environmental release. Depending on the kind of interactions that substances have with fabrics, two kinds of release can occur: molecular and particle release [36].

For instance, UV absorbers are added to prevent polymer and dye degradation when fabrics are exposed to UV radiation, simultaneously obtaining an improved dye fastness [50]. Interactions with fabrics of some UV-absorber and direct dyes, such as in coloring of cellulosic fibers, are non-covalent, i.e. include van der Waals, electrostatic, induction, and solvophobic interactions [51]. Other examples are biocides, added during the storage, which are just adsorbed to the textile material. Compounds that are loosely bound to the fabrics would have mostly molecular release, on the other hand substances that are covalently bonded will have fabric mediated release, such as for the reactive dyes [40].

The release of chemical can also be triggered by humidity, high temperature, UV-radiation (sun light), physical stress (friction) and or sweat. Although the human skin is a barrier against environmental pollutants (stratum corneum), it has been shown that during perspiration some textile dyes can migrate from the fabrics and penetrate into the skin [52]. Furthermore, dyes have been extracted from cotton fabrics by
surrogate sweat showing differences according to application methods, composition and pH of the sweat [53].

1.4.1 Human exposure

We spend most of our lives in intimate contact with clothes. Despite several substances with known toxicity have been detected in textiles, studies about health effects associated to textile exposure are mainly focused on contact dermatitis [54–56]. Evidences of exposure to chemicals contained in textile materials have been investigated for decades. Human exposure to chemicals in textiles is possible through inhalation, ingestion and dermal absorption [49]. In the late 1970s, a mutagenic metabolite of tris(2,3-dibromopropyl) phosphate, used as flame retardants in children pajamas, was found in the subject’s morning urine after they had slept in pajamas impregnated with tris(2,3-dibromopropyl) phosphate [57]. Recently, it has been shown that forestry workers, using tick-proof pants containing the insecticide permethrin, had high levels of a metabolite of the insecticide in their urine [58]. Both these exposures are most likely due to skin adsorption of the chemical from the garments.

Exposure by inhalation is possible for volatile compounds such us perchloroethylene used as dry-cleaning solvents [59]. Clothing has also been identified as a significant source of inhalable allergens, such as dust particles and cat allergens [60,61].

Another possible exposure pathway is by ingestion, which can be significant, particularly for young children, when mouthing the fabric. An example of this exposure has been estimated for azo dyes extracted from textiles by saliva surrogate [62]. However, the estimated amount of azodyes in saliva simulant were only a fraction of the total amount detected in the fabrics, a percentage between 0.6 and 8%.
Dermal absorption is by far the most prominent pathway for chemicals contained in textiles, since clothes are in prolonged and close contact with the skin [40]. *In vitro* experiments have showed the absorption from fabric into the skin of ethylene oxide (fumigant) [63], glyphosate (herbicide) [64] and malathion (insecticide) [49].

Textiles may also mediate exposure of chemicals acquired post purchase. As an example, higher serum levels of polychlorinated biphenyls (PCBs) were found in a woman who laundered agricultural work clothes [58]. Another example is the dermal exposure to airborne phthalates, which is reduced if the skin is covered by non-contaminated clothes, acting as a barrier. However, if clothes have previously been exposed to phthalates, the resulting exposure is increased [65].

### 1.4.2 Environmental release

Along with human exposure, environmental concerns rise from chemicals being released during laundering. Substances can leach from the textiles into waste water during domestic washing and if they are not removed from the waste water stream in the wastewater treatment plant (WWTP), they are released into the environment.

Textile washing has been estimated to account for approximatively half of the total environmental release of nonylphenol ethoxylate and its degradation product nonylphenol [66]. Our research group also showed that concentrations of benzotriazoles, benzotriazoles, and quinolines decreased in textile material, t-shirts, after five to ten laundry washing cycles [67]. Moreover, in a collaborative work carried out between Umeå University, The Swedish Environmental Protection Agency and Stockholm University, seventy-two compounds were detected in the laundry waste. Some of these were not completely removed in Swedish
WWTP effluents. In the mentioned study, compounds released in large amount were phthalates, organophosphates and formaldehyde [68].

1.5 Analytical methods

Limited regulations for chemicals used in the multistep textile manufacturing process, together with sparse analytical data, make textile materials important targets for chemical analysis. In order to assess the presence of low concentration of several contaminants in complex matrices, highly selective and reliable multiple-class analytical methods are required. Such preliminary investigations are fundamental steps towards the human and environmental risk assessment, providing the information necessary to take actions to minimize such effects. The sample preparation for textile materials consists of leaching by an organic solvent, and a successive cleanup step. The main focus of this work was on the cleanup procedure and therefore the leaching is not discussed in details.

1.5.1 Sample preparation

Sample preparation is crucial in the development of any analytical method. To successfully perform simultaneous analysis of several organic micro-pollutants prior the use of chromatographic method, sample purification of extracts as well as analyte pre-contraction from liquid samples are required. Enrichment and isolation of analytes from interfering compounds present in the matrix [70] are often performed in a single step by solid phase extraction (SPE). Once a SPE sample treatment procedure is properly optimized, the improvements in the limits of detection and the reduction of interfering compounds results in higher precision and
accuracy of the method [71]. Finally, SPE procedure can be easily automated thus allowing a theoretical high throughput and reducing the costs of large scale investigations [72].

The choice of the appropriate SPE sorbent depends on the nature and number of target compounds. Different kind of interactions might be established between analytes and the sorbent depending on their different chemical and physical properties, for instance lipophilicity and/or acidity [71,73]. The analyte interactions established with the sorbent can be selectively disrupted using a suitable mobile phase [74].

Several kinds of sorbents can be used for SPE, for instance traditional silica based reverse phases (C8, C18), normal phase (silica, alumina), size exclusion, ion exchange, immune affinity, molecular imprinted polymers (MIP) and carbonaceous sorbents [72,73,75].

1.5.2 Carbonaceous sorbents

Carbonaceous sorbents are a large family of sorbent materials commonly used in filtering and purification. Carbonaceous sorbent can be classified into two groups according to Rosalind Franklin (1920-1958), who is well known for her important role in discovery of the structure of DNA. What is less known is her study of coals, carbon, and graphite. Her most remarkable contribution in this research field was identifying the fundamental distinction between graphitizing and non-graphitizing carbon. The two kinds of carbon materials differ in the structural unit organization, which is approximatively parallel in the former and random in the latter, as it is shown in figure 3 [76].
Activated carbons are obtained from non-graphitizing carbon and were the first carbonaceous materials used to retain medium-to-low polarity organic compounds from water [77] and by far, the most used synthetic sorbent. Their adsorption properties are characterized by a large number of low-volume pores, able to increase the specific surface area up to a few thousand square meters per gram [78]. Starting material and activation process, the latter obtained by thermal or chemical method, will define the characteristics of the sorbent in terms of porosity (space) enclosed by carbon atoms, pore size distribution and specific surface [79]. Although activated carbons find several industrial applications, for instance, in potable and waste water treatment and air/gas purification, the use of these materials are not suitable for SPE due to low recovery and irreversible adsorption of even relatively small organic compounds [77].

1.5.2.1 Graphitized Carbons

In this thesis graphitized carbon black (GCB) sorbents have been used to improve cleanup of target analytes from textile extracts and to sample and concentrate these from water samples.
Graphitic materials are made of intertwined sheets of sp² hybridized carbon atoms in hexagonal arrangement. These materials are not amorphous, however, their atomic structures differ from graphite since the organization of successive layers is not regular [80]. Figure 4.

![Atomic structures of (a) graphite and (b) graphitized carbon](image)

**Figure 4:** Atomic structures of (a) graphite and (b) graphitized carbon [81]

Among graphitizing carbons, Graphitized Carbon Blacks (GCBs) are a family of relatively homogeneous and non-porous materials investigated as sorbents since 1950’s [82]. GCBs were thoroughly investigated for their use as stationary phase in gas-chromatography by Kiselev, Horvath, Guiochon and Bruner [83–86] and SPE for aqueous samples [87] showing outstanding ability in retaining polar and hydrophilic compounds [88]. Porous graphitic carbon (PGC) is characterized by the same atomic structure as GCBs, however, it is obtained from graphitization of a synthetic polymer, made from phenol and hexamine, around silica particle used as template, which subsequently are dissolved by a potash solution before graphitization [80].

In contrast to the PGC, GCB materials are not suitable for high performance liquid chromatography (HPLC) because they have a low resistance to mechanical stress [80]. Although PGC is a different material
compared to GCB, their retention behavior shows some similarity, and with the introduction of PGC the high potential of graphitized carbon sorbents to retain polar compounds was confirmed. The retention of non-polar compounds can be explained in terms of dispersive interactions, e.g., Van der Waals and London forces. These interactions occur between the analyte molecule and the large number of aromatic carbon atoms of the GCBs graphitic structure. For instance, long chains in fatty acid methyl esters will result in increased retention compared to short chains [89]. The chromatographic behavior of substituted aromatic rings is explained by $\pi-\pi$ interactions, and the retention of flat molecules is attributed to interactions with the planar graphitic surface of the GCB [90, 91]. For instance, as reported in Figure 5, the stronger retention of $p$-xylene compared to its structural isomer ethylbenzene, is explained by the molecular geometry of the latter giving rise to sterically hindrance of the $\pi-\pi$ interactions for ethylbenzene.

**Figure 5:** *Flat molecules have more contact with the planar graphitic surface [90]*

Retention of polar compounds on GCB have been explained by the so-called “polar retention effect on graphite” (PREG) theory, which is based
on dipole-induced dipole interactions. The atoms in the graphitic grid are sp\(^2\) hybridized forming an electronic cloud above its surface. When a charge, positive or negative, approaches the graphitic surface the electron cloud of the graphitic structure is attracted or repelled [80], Figure 6.

**Figure 6:** Polar retention effect on graphite (PREG) theory for retention of polar compounds [80]

Another theory was suggested by Hanai, whose mathematical model predicted an electron deficient area in the middle of graphitic planes, being close to neutrality, while the edges of the planes would have an excess of electron resulting in a negative charge. Thus, different interaction zones would be possible in the graphitic plane for neutral and charged compounds [92], Figure 7.

**Figure 7:** Different interaction zones according to the Hanai theory: grey zone neutral compounds, in blue and red positively and negatively charged compounds, respectively [80]
Although the above-mentioned interactions are similar in GCBs and PGC, the nature of the surface varies for the different kind of graphitic materials [93]. Features present on GCBs sorbent results in unexpected strong retention of anionic compounds and, in a few cases, chemisorption. This behavior was attributed to the presence of oxygen complexes with structures like hydroquinone, quinones, chromene and benzpyrylium [94]. These impurities on the surface give GCBs ion exchange properties [95]. These heterogeneities of the GCB surface, under suitable conditions, results in mixed-mode chromatography, thus allowing analytes fractionation by highly selective desorption [96]. The multitude of possible interactions has been exploited to obtain cleanup and selective elution, for instance in the fractionation of estrogen conjugates in late-pregnancy fluids [97].

Recently GCBs became very popular in QuEChERS (quick, easy, cheap, effective, rugged and safe) methods where small amounts of GCB gives the effective removal of natural pigments in the sample pretreatment step. GCBs find a large number of applications with this kind of sample preparation scheme [98]. Other current uses of GCBs are in thermal desorption analysis for environmental applications [99], lipid fractionation [100] and small polar peptide analysis [101].

1.5.3 Target analytes

In this work, target screening methods of textile samples were applied for the determination of compounds belonging to four different chemical classes: quinolines, benzotriazoles, benzothiazoles and nitroanilines, (Table 1).

The main sources for the production of quinolines are coal, tar, and petroleum. These compounds have a wide range of industrial applications,
such as in pharmaceuticals, wood treatment, food additives, cosmetics etc. [102–104]. In textile production, quinolines are extensively used as dye precursors [105]. Quinolines are relatively soluble in water and have been detected in ground water, as well as in lake, and marine sediments [106,107]. The carcinogenic activity of some quinoline derivatives has been demonstrated in animals, but no human studies are available [108].

Benzotriazoles and benzothiazoles are used in several industrial applications, such as vulcanization accelerators in rubber production, corrosion inhibitors, etc. In textile production they are used as components of dispersive dyes and as UV stabilizers [109–113]. These classes of compounds are often detected in wastewater treatment plants effluents [114]. The widespread use of these chemicals and their toxicity to aquatic organisms make them important environmental pollutants [115].

Besides the dye production, nitroanilines are used in the synthesis of pharmaceuticals and polymers, as well as in the production of rubber, pesticides, and explosives [116]. In addition to their toxicity, some nitroanilines show bio-accumulative properties and are potentially carcinogenic [117].

Figure 8: Structures of (a) benzothiazole, (b) meta-nitroaniline, (c) benzotriazole and (d) quinoline.
Table 1: Benzotriazole, benzothiazole, nitroaniline, quinoline and their derivatives included in these studies.

<table>
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<th>Abbreviation</th>
<th>CAS</th>
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1.5.4 High resolution mass spectrometry based screening

Triple quadrupole (QqQ) and linear ion trap (QIT) mass spectrometers are commonly used for quantification purposes due to their high sensitivity, high selectivity, and the ability to perform fast scans. However, methods performed with these instrumentations show some limitations:

- It has been demonstrated that the monitoring of one transition alone for a specific compound might result in false positive identification, for this reason many guide lines in validation processes require the monitoring of two transitions [118]. Under the constraint that at least two transitions are needed in Selected Ion Monitoring (SRM) methods, typically, “only” 100-150 compounds can be acquired in a single chromatographic run [119];
- For some compounds, non-specific transitions such as the neutral loss of H₂O or CO₂ might occur, which are less specific transitions for analytes and increase the noise levels [119];
- Ions may show only one transition and/or the second transitions may not have a sufficient signal intensity, resulting in relatively
high limit of quantification (LOQ), as in the case of sucralose [120].

High resolution mass spectrometry (HRMS), in some case, is a promising solution to overcome these limitations. The compound identification is obtained exploiting the high-resolution power of these kind of mass spectrometers operating in full scan mode. Theoretically, an “unlimited” number of compounds can be determined in each sample, in contrast to SRM [119]. However, with high resolution full scan, false positive identifications can occur for co-eluting and isobaric compounds, i.e. for N-nitrosomorpholine in wastewater samples [121].

HRMS plays an important role in the discovery of new hazardous chemicals by applying non-target screening in combination with data mining methods. With hybrid mass spectrometers it is possible to obtain MS/MS data in combination with full scan spectra, which can be further used in the identification process [122].

1.5.4.1 LC/ESI coupled to hybrid linear ion trap Orbitrap® mass spectrometer

In the Orbitrap®, hybrid linear ion trap mass spectrometer, two mass analyzers are connected in tandem to obtain MS/MS spectra: the quadrupole and the Orbitrap®, Figure 9a.
After the production of ions in the ion source (i.e. electrospray ionization ESI), the ions are guided to the first mass analyzer, the quadrupole mass filter. Subsequently, ions are collected in the C-trap (a curved linear trap) and sent to the collision cell or directly to the Orbitrap, according to the desired scan mode. When ions are injected into the Orbitrap, they are electrostatically trapped while rotating and oscillating, in z and r directions, along the central electrode. The mass over charge ratio ($m/z$) of the ion is related to its oscillation along the z-axis, and frequency signals from the ions are collected and converted to a mass spectrum using Fourier transformation [124]. **Figure 9b.** Resolution power depends on the time that the ions spend in the Orbitrap, longer time results in higher resolution.
The possible scan modes in the Orbitrap® are [125]:

- Full scan. Ions travel from the quadrupole, operating in RF mode, to the C-trap that injects ions in the Orbitrap.
- Selected ion monitoring (SIM). Ions with selected $m/z$ are filtered from the quadrupole, collected in the C-trap, and injected into the Orbitrap.
- Data Dependent Acquisition (DDA). The instrument performs a full scan in a selected $m/z$ range. Subsequently, the most abundant ions, or a set of selected ions defined by a priority list (typically 5 ions), are individually filtered by the quadrupole. Successively, they are collected in the C-trap, sent to the collision cell, re-sent to the C-trap, and finally injected into the Orbitrap.
- Data Independent Acquisition (DIA). Ions are filtered from the quadrupole with a wide isolation window (typically 20 $m/z$), fragmented in the collision cell, and successively injected into the Orbitrap.
- Parallel Reaction Monitoring (PRM). This scan mode is the most similar to Selective Reaction Monitoring (SRM) and is usually
performed for quantification purposes. The quadrupole individually filters selected $m/z$, which are collected in the C-trap, sent to the collision cell, re-sent to the C-trap, and finally injected into the Orbitrap. The main difference to MRM is that all product ions are obtained simultaneously in a single MS/MS scan.
2. Results and discussion

The sample preparation is an important step in the analytical chain aimed at removing interfering compounds and/or concentrate the sample prior to the subsequent analysis. In gas chromatography, when the injection includes a high temperature volatilization step, the removal of interfering compound could minimize the risk of artifact formation. In the case of textile extracts, artifacts could be generated from breakdown of dyes in the injection port. Sample preparation can also be useful to concentrate compounds present in samples at trace levels. Determining identities and concentrations of chemicals in textile materials is necessary to be able to estimate dermal exposure and assess associated risks. Monitoring of known and unknown substances in textiles is an important basis for improving and expanding regulations of chemicals in textiles.

In Paper I, a GCB-based cleanup procedure was applied in order to remove co-extracted dyes prior to the determination of quinolines and nitroanilines in textile material analysis. In Paper II, a permeation study of benzothiazole, a commonly detected chemical in clothing textiles, was performed to demonstrate skin absorption/penetration. Additionally, a rough estimation of the carcinogenic and non-carcinogenic risks associated with benzothiazole was made for a hypothetical tight worn t-shirt. In Paper III, GCB was used to sample and concentrate textile related compounds from water matrices. The developed method was subsequently applied to the analysis of the effluents from three wastewater plants located in Stockholm. With the basis of the large number of possible different chemicals detected in various textile materials, a strategy towards suspect and non-target screening was suggested in Paper IV.
2.1 Paper I - Cleanup of textile extracts using graphitized carbon black (GCB)

When textiles are extracted by an organic solvent, dyes as well as other contaminants, contained in the samples, are simultaneously leaching from the fabrics. GCB is commonly used as SPE stationary phase due to its ability of strongly retaining analytes by different kinds of interactions (including van der Waals, London dispersive forces, π-π interactions, PREG, anion-exchange adsorption site). Thus, when suitable elution conditions are found, it is possible to obtain a class selective release of analytes resulting in a more effective cleanup of the sample, if compared to traditional reversed phase sorbents (e.g. C18) [126].

For this reason, GCB is used in QuEChERS as a dispersive solid phase extraction material to remove pigments and chlorophylls from extracts of vegetables in pesticides analysis. Due to the much stronger interactions of large and flat molecule with the graphitic surface, chlorophylls (colorful pigments) are strongly retained by GCB even when using organic solvents such as acetonitrile, thus resulting in selective elution of pesticides and ineffective elution of chlorophylls [82].

Applying the same principle as for QuEChERS, GCB was used in solid phase extraction to remove co-extracted dyes from textile materials, while simultaneously obtaining elution of the target analytes, which have lower interaction with sorbent compared to dyes.

A pilot discoloration test was performed on a number of textile extracts to investigate the effectiveness of the dyes retention on GCB. Each textile sample was extracted by 2x10 ml of DCM, which was concentrated to a volume of approximately 1 ml. In order to remove the dyes from the
sample, the extract was percolated through a 250 mg Carbograph® 1 SPE-cartridge, preconditioned with DCM, and collected in the first fraction. Further nine 1 mL fractions of elution solvent (DCM) were passed through the cartridge and collected in separate test tubes. The effectiveness of dyes cleanup with GCBs is shown by the results of the discoloration test presented in Figure 10.

![Figure 10](image)

**Figure 10:** *Discoloration test of a textile extract, to the left, eluted as ten 1 ml dichloromethane fractions, to the right, from a GCB Carbograph 1 SPE cartridge.*

A compromise between high recoveries and cleanup efficiency had to be obtained for nitroanilines and quinolines. Two GCB materials were tested, namely Carbograph® 1 and Carbograph® 4, packed in SPE cartridges in two different amounts, 250 and 500 mg. The weakest sorbent combination, 250 mg of Carbograph® 1, was found to provide the best compromise between efficiency of the cleanup and recovery of the analytes. It is known that pure methanol (MeOH), acetonitrile (ACN) and dichloromethane (DCM) have weak elution strength on GCB. In previous studies mixture of DCM:MeOH (95:5, v:v) was recommended for elution of some pesticides and other polar compounds [127]. Furthermore, chloroanilines have been eluted from GCB using acidic conditions [128]. Thus, neutral and acidic mixtures of dichloromethane and methanol (95:5, v:v) were also tested. However, this approach did not provide significantly higher recovery yields in the SPE step for the investigated compounds as it is
reported in Figure 11. Instead, the best SPE conditions for the elution were obtained by using 5 ml of pure DCM and a mixture of DCM with 20 % of toluene, Figure 12.

**Figure 11:** Recovery percentages for nitroanilines and quinolines using 5 ml of DCM:MeOH (95:5, v:v) and 10 mmol/L trifluoroacetic acid in DCM:MeOH (95:5, v:v)

**Figure 12:** Recovery percentages for nitroanilines and quinolines using pure DCM (red) and DCM:Tol (80:20)(blue)

Using these SPE-conditions, it was evident that quinolines and nitroanilines exhibited a different chromatographic behavior on GCBs. In addition to dispersive forces, which can explain the retention mechanism
for quinolines, the electrostatic interactions play a significant role in the retention mechanism of nitroanilines. In a previous investigation on another graphitic sorbent (PGC) [91], toluene was used to efficiently elute nitro-aromatic compounds. The key for elution of nitroanilines was the percentage of toluene in the elution solvent. In our case the addition of 20% (v:v) of toluene to pure DCM resulted in selective elution of all the investigated compounds and strong retention of dyes which were still absorbed to GCB. Two examples of textile extracts before and after cleanup with GCB SPE are shown in Figure 13. In the optimized conditions five milliliters of elution phase (DCM:Tol, 80:20 v:v) were sufficient for the elution of all the investigated compounds. The method was used to determine the concentrations of nitroanilines and quinolines in a number of textile samples. Concentrations of nitroanilines in cloth samples were found to be 2/3 order of magnitude higher than quinolines.

Figure 13: Textile extracts before (a) and after GCB SPE cleanup (b)
2.2 Paper II - Permeation study and risk assessment of benzothiazole in textiles

In Paper II the possible absorption in / permeation through skin of textile related was investigated. To determine concentrations in textile materials is the first step towards understanding possible exposure that may be associated with chemicals present in textiles, but it is also essential to investigate the possible transfer of these chemicals from the textile material to the skin.

Benzothiazole (BT) was chosen as model compound, since it has been frequently detected at relatively high concentrations in textiles. Moreover, its water solubility is compatible for permeation studies. BT acute toxicity effects are characterized by depression of central and respiratory systems, liver and kidney toxicity. Furthermore, BT is a possible skin allergen [129].

The permeated percentage of BT through the skin was estimated by using a Strat-M transdermal diffusion membrane, a synthetic membrane mimicking the different skin layers (*straum corneum*, epidermis and dermis), in *in vitro* experiments, together with Franz cells and flow-through diffusion cells. Both systems consist of a donor and a receiving chamber, separated by the membrane. In Franz cells, the receiving chamber is a static system, while in flow-through diffusion cells it is dynamic, with the receiving fluid being constantly replaced by a continuous flow. Although the Franz cell equipment meets the pharmacopeia standards, the diffusion cell could be more suitable to study the permeation of compounds with low water solubility, which might be the case for many textile contaminants.
The aim of this study was to estimate the percentage of benzothiazole absorbed on and permeated over time through the human skin mimicking membrane. Concentrations levels in the donor chamber were adapted to the multiple fractions taken from the receiving chamber of the Franz and as well as the diffusion cells, combined with the limit of detection of the analytical method.

The *in vitro* study showed that a substantial percentage of benzothiazole was transported through the membrane into the receiving fluid. Additionally, a fraction of BT was found absorbed in the skin-mimicking membrane. The experimentally determined permeation was used in a simplified model, **Equation 1**, to estimate dermal exposure, where the different terms in the equation are specified in **Table 2**.

\[
\text{Exp}_{\text{derm}} = \text{Conc}_{\text{cloth}} \times d_{\text{cloth}} \times A_{\text{skin}} \times F_{\text{contact}} \times F_{\text{mig}} \times F_{\text{pen}} \times T_{\text{contact}} \times \frac{n}{BW}
\]

(Eq. 1)

**Table 2**: Description of terms in Equation 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc_{cloth}</td>
<td>T-shirt BT median concentration</td>
<td>g/g</td>
</tr>
<tr>
<td>d_{cloth}</td>
<td>T-shirt mean areal density</td>
<td>mg/cm²</td>
</tr>
<tr>
<td>A_{skin}</td>
<td>Mean exposed skin area (adult)</td>
<td>cm²</td>
</tr>
<tr>
<td>F_{contact}</td>
<td>Fraction of skin contact area</td>
<td>unitless</td>
</tr>
<tr>
<td>F_{mig}</td>
<td>Fraction of substance migrating to skin</td>
<td>%</td>
</tr>
<tr>
<td>F_{pen}</td>
<td>Fraction of penetration inside body</td>
<td>%</td>
</tr>
<tr>
<td>T_{contact}</td>
<td>Contact duration between skin-textile</td>
<td>day</td>
</tr>
<tr>
<td>N</td>
<td>Mean number of events per day</td>
<td>day⁻¹</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
<td>kg</td>
</tr>
</tbody>
</table>

The BT concentration used to estimate the risk assessment was the median concentration obtained from eight t-shirt analyzed by Luongo et al (4.0x10⁻⁴ g/g). The calculated dermal dose was used to assess the associated carcinogenic and non-carcinogenic risks related to wearing a
hypothetical t-shirt containing benzothiazole. The dermal exposure of benzothiazole from a tight t-shirt contact was calculated to be 1.36 µg kg\(^{-1}\) day\(^{-1}\), for the worst assumption. The associated non-carcinogenic risk or hazard quotient (HQ) was calculated by comparing the obtained dermal exposure with the reference dose (RfD) of benzothiazole (5 µg kg\(^{-1}\) day\(^{-1}\)) [129]. The HQ was estimated to be 0.27, indicating a risk below the acceptable daily intake.

The carcinogenic risk was assessed by using a value suggested from literature: the slope factor of 2-mercaptobenzothiazole (6.34x10\(^4\) mg kg\(^{-1}\) day\(^{-1}\)) [129]. The resulting risk was estimated to be 0.86x10\(^{-6}\), thus less than one per million cancer risk level, which is in the acceptable range according to international standards. It should be pointed out that the migration factor used in this work was not possible to determine experimentally and the RfD is related to dietary exposure, not skin exposure.

### 2.3 Paper III - Water samples analysis

Another concern regarding chemicals in textiles is the possible release to the environment. We have shown that the concentration of quinolines, benzothiazoles, and benzotriazoles present in clothing textiles decreases during 5 to 10 washes [67]. Previously, decreasing concentrations were estimated by analyzing textile materials before and after washing. The aim of Paper III was to develop an analytical method to sample and concentrate these compounds from a water matrix and further to investigate the possible release to the environment. The developed method was subsequently applied to the analysis of effluents from three wastewater treatment plants located in Stockholm.
Based on previous experiments, described in Paper I, SPE with GCB sorbent was the method of choice, as it demonstrated quantitative elution of quinolines and nitroanilines. Taking into account the aqueous matrix of the samples, adjustments were required. Previously, the elution of these compound groups was performed by water-immiscible solvents (dichloromethane and toluene). For this reason, a water removal step needed to be implemented before the SPE elution phase. Moreover, breakthrough and/or incomplete desorption when using large water volumes had to be investigated.

Different MQ-water volumes (500 and 2000 ml) were spiked with the target analytes and percolated through two SPE-cartridge (250 mg of Carbograph®) connected in series, as in Figure 1 of Paper III. Before elution, a water removal step was performed by vacuum drying the SPE sorbent and washing it with 0.5 ml of MeOH. The first cartridge was eluted with two separate fractions of 5 ml DCM:Tol (80:20, v/v) each. The two fractions were analyzed separately to determine incomplete desorption. The second cartridge, used as back-up to determine breakthrough, was eluted with one fraction of 5 ml DCM:Tol, (80:20, v/v). Dinitroanilines, compounds with two nitro groups on the aromatic ring, were detected only in the first fraction of first SPE cartridge, but not in the second fraction, and nor in back-up cartridge. This suggested that dinitroanilines were still adsorbed on GCB, highlighting a different behavior compared to the previous studies in Paper I, where DCM:MeOH (80:20, v:v) was able to elute these compounds. This difference was attributed to the aqueous sample matrix.

To obtain satisfactory recoveries for all nitroanilines (i.e. a yield >70%), the percentage of toluene was increased from 20 to 50 % in the 5 ml elution
solvent. Furthermore, DCM was replaced by methanol, which helped in the following evaporation step since it produces an azeotrope with toluene. Quinolines showed a different retention mechanism in water matrix samples compared to nitroanilines. In this case DCM was found to be the best eluting solvent, giving a more selective elution and decreasing the sample signal background in the subsequent MS analysis.

The same set-up and SPE-conditions used for the nitroanilines were also applied to benzotriazoles and benzothiazoles. High and reproducible recoveries from MQ-water were obtained for all compounds except 2-(benzotriazol-2-yl)-4-methylphenol (UV-P). This compound was also found in the second 5 ml fraction from the first SPE cartridge. As a consequence, the volume of elution solvent was finally set to 10 ml, and the possible sample volume could not exceed 2000 ml.

The method was then evaluated using a spiked wastewater matrix. Since was not possible to find blank wastewater sample the spiked levels were substantially higher than the amounts present in the samples and thus blank subtraction was performed. When applied to WWTP effluents analysis, breakthrough was found to be substantially higher compared to MQ water matrix. This lead to a decrease in the maximum sample volume to 100 mL and the need of larger amounts of GCB sorbent (500 mg). However, the use of 500 mg GCB lead to a decreased recovery of UV-P (≈20%), which was once again detected in the second 10 ml fraction of the top cartridge, leading to an increase in the required elution volume. Indeed, the final conditions suggested for target analysis of wastewater treatment plant effluents were: 100 mL water, sampled and concentrated on a 500 mg of Carbograph® 1 SPE cartridge, eluted with a total volume of 20 ml methanol/toluene (50:50, v/v).
In a pilot study, the method was applied on effluents from three WWTPs located in the Stockholm area: Henriksdal, Bromma and Käppala. In this study 1-H-benzotriazole, 4/5-tolyltriazole and UV-P were detected and quantified. The concentrations were in the same range as those previously reported from studies performed in other countries, Table 3 [130].

Table 3: Concentrations of 1-H-benzotriazole, tolyltriazole and UV-P in analyzed WWTPs located Stockholm (ng/L), n=3.

<table>
<thead>
<tr>
<th>Effluent sample</th>
<th>1-H-benzotriazole</th>
<th>Tolyltriazole</th>
<th>UV-P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Henriksdal</td>
<td>493 (+/- 76)</td>
<td>638 (+/- 48)</td>
<td>89 (+/- 33)</td>
</tr>
<tr>
<td>2. Bromma</td>
<td>326 (+/- 30)</td>
<td>1148 (+/- 160)</td>
<td>53 (+/- 31)</td>
</tr>
<tr>
<td>3. Käppala</td>
<td>622 (+/- 220)</td>
<td>743 (+/- 174)</td>
<td>75 (+/- 51)</td>
</tr>
</tbody>
</table>

2.4 Paper IV - Strategies towards suspect and “non-target” screening of textile samples

There are much more chemicals present in textile materials than the four compound classes investigated so far by target analysis methods. The extensive use of chemicals in the textile production, the information lost along the textile manufacture chain, together with the possible human exposure as well as environmental release, make textiles an important target for research of new possible chemical hazards.

Although the acquisitions performed in this study allows a non-target screening methodology, i.e. not involving the use of any database, the focus was put on suspect screening. In this study, the strategy towards the discovery of new unknowns will be referred to as non-target screening approach.
The discovery of unknown contaminants in samples is by far the most challenging task for an analytical chemist. In this perspective, the use of high-resolution mass spectrometers (HRMS) is a big advantage. With this kind of instrumentation, the determination of exact masses can be used for molecular formula prediction and compound identification.

When the mass spectrometric resolving power is sufficiently high, the molecular formula can be predicted from the measured ions. The molecular formula for benzothiazole is \( \text{C}_7\text{H}_5\text{NS} \) with a calculated exact mass of 135.014271 Dalton. With a 5 ppm mass accuracy the accepted range of \( m/z \) would be between 136.0213 and 136.0227 Dalton. Searching the ChemSpider database, the number of compounds in this mass range is 47, in which the majority are isobaric structures. If we widen the accepted mass range, more compounds would be included in this list. For example, increasing the mass accuracy first from 5 to 50 ppm and then to 500 ppm, the number of possible compounds increase from 47 to 364 and then to 5764, respectively. Thus, by using high resolution mass spectrometers it is possible to obtain the molecular formula and get a limited number of suggested compounds, which is beneficial for compound identification.

According to the procedure described by Luongo et al [126], twenty-four textile sample extracts were analyzed with a Q-Exactive Orbitrap® instrument, a hybrid high resolution mass spectrometer. Samples were analyzed in positive and negative ion mode, using data dependent acquisition (DDA) with and without activated inclusion list. The inclusion list was a selection of exact masses for compounds from the EUs list of Substances of Very High Concern (SVHC), Swedish Environmental Protection Agency and known textile contaminants. The full scan mass range was set to 100 to 1000 \( m/z \) at a mass resolution of 120,000. The
resolution of the MS$^2$ scans was set to 30,000, and a collision energy of 30 eV.

While advantageous for compound identification, using the mass spectrometer in high resolution mode produces large data sets which are impossible to handle without a dedicated software. In Paper IV the Compound Discoverer 2.0 (Thermo Fisher, Massachusetts, USA) software was used to process the raw-data, extract the ions, and generate suggestions based on a specified work flow. This included selection of spectra, retention time alignment, and isotopic pattern identification. Masses were matched against two data bases: ChemSpider and MzCloud. The former generates suggestions based only on the exact mass value, whilst the latter gives a percentage score of the match considering MS$^2$ data as well. ChemSpider includes a larger number of compounds than MzCloud. The software produced four lists obtained from four datasets: i.e. from running positive top-5, negative top-5, positive suspect and negative suspect. A large number of suggestions was obtained, which needed further filtering.

For the negative top-5 dataset alone, the suggestions after software processing were more than 200,000, and even more for positive ion mode. These decreased to approximately 10% (20,000) when excluding suggestions for peaks with intensities lower than 3 times the blank signals, not present in duplicates and/or no MS$^2$ data available.

A molecular weight prediction model was applied as an added filter to further increase the certainty of identification. The retention time of compounds within a molecular weight ranging from 119 to 447 Dalton was used to construct a model with HPLC retention time as a linear function of the molecular weight. The error mean square root was calculated, yielding a retention time window of four minutes. Thus,
filtering was done by only keeping suggested compounds with a difference between the calculated and experimental retention time within +/- 2 minutes of the measured retention time. After this step, the number of suggested compounds was reduced approximatively to 1% (2,500).

Although the number of suggested compounds was heavily reduced at this stage, it was neither time efficient nor economically realistic to test 2,500 reference substances. The identification confidence was still low and more suggestions were given by the other datasets obtained from the different scan modes. Further filtering was made by only keeping suggestions from ChemSpider if more than 500 references were found in the database, and mzCloud suggestions were kept only if the matching score was higher than 70%.

For the top-5 negative dataset the number of suggestions from the ChemSpider database was reduced to 25 after all filtration steps. These suggestions were finally filtered with regards to the compounds relevance/probability to be present in textile samples and standard availability.

The finally suggested compounds were identified by analysis of reference standards on the LC/MS system and matching retention times, exact masses, fragmentation and isotopic patterns by visual inspection. With this strategy for non-target screening approach it was possible to identify nitrophenols, phthalates, organophosphates and acridine in the analyzed samples. Through the suspect screening approach, the presence of benzothiazoles, benzotriazoles, nitroanilines and quinolines were confirmed in the samples. Occurrences of identified compounds are reported in the Table 3 and Table 4.

When performing non-target analysis one has to be aware of the possible discrimination of relevant compounds due the filtration steps applied on
the dataset, losses in the cleanup that often is necessary to apply prior to instrumental analysis with the LC/HRMS system, as well as the analyte dependent ionization in the mass spectrometer ion source.
Table 3. Compounds identified by suspect screening.

<table>
<thead>
<tr>
<th>Name</th>
<th>Measured m/z</th>
<th>Theoretic m/z</th>
<th>Mass Accuracy (ppm)</th>
<th>Formula</th>
<th>Fragmentation pattern (m/z)</th>
<th>RT</th>
<th>Confirmed in sample no</th>
<th>Detection frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Positive ion mode</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinoline</td>
<td>130.0653</td>
<td>130.0657</td>
<td>3.08</td>
<td>C9H7N</td>
<td>103.0545 – 95.0493</td>
<td>6.17</td>
<td>1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,20,22,23,24</td>
<td>19</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>136.0216</td>
<td>136.0221</td>
<td>3.68</td>
<td>C7H6NS</td>
<td>109.0107 – 65.0388</td>
<td>6.32</td>
<td>1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,20,21,22,23,24</td>
<td>22</td>
</tr>
<tr>
<td>5-methylbenzotriazole</td>
<td>134.0714</td>
<td>134.0718</td>
<td>2.98</td>
<td>C7H7N3</td>
<td>95.0492 – 105.0448</td>
<td>4.86</td>
<td>7,9,22,24</td>
<td>4</td>
</tr>
<tr>
<td>UV-P</td>
<td>226.0980</td>
<td>226.0980</td>
<td>0.00</td>
<td>C13H12N3O</td>
<td>79.0543 – 120.0557</td>
<td>13.36</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>UV-234</td>
<td>448.2380</td>
<td>448.2389</td>
<td>2.01</td>
<td>C30H29N3O3</td>
<td>370.1913 – 119.0856</td>
<td>19.28</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td>Methylquinoline</td>
<td>144.0808</td>
<td>144.0813</td>
<td>3.47</td>
<td>C10H9N</td>
<td>115.0544 – 91.0543</td>
<td>6.94</td>
<td>3,4,6,11,12,13,14,15,20,22,23,24</td>
<td>12</td>
</tr>
<tr>
<td>Diisobutyl phtalate</td>
<td>279.1586</td>
<td>279.1597</td>
<td>3.94</td>
<td>C16H12O4</td>
<td>149.0234 – 57.0702</td>
<td>13.77</td>
<td>3,4,8,10,15,17,22,24</td>
<td>9</td>
</tr>
<tr>
<td><strong>Negative ion mode</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-mercaptobenzothiazole</td>
<td>165.9782</td>
<td>165.9785</td>
<td>1.81</td>
<td>C7H6NS2</td>
<td>134.0060 – 57.9755</td>
<td>5.82</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>2-bromo-4,6-dinitroaniline</td>
<td>259.9313</td>
<td>259.9307</td>
<td>-2.31</td>
<td>C6H4BrN3O4</td>
<td>229.9331 – 199.9349 - 78.9190</td>
<td>8.56</td>
<td>1,2,3,4,5,6,10,11,12,15,20,21,23,24</td>
<td>14</td>
</tr>
<tr>
<td>2,6-dichloro-4-nitroaniline</td>
<td>204.9570</td>
<td>204.9572</td>
<td>0.98</td>
<td>C6H4Cl2N2O2</td>
<td>174.9588 – 59.0136</td>
<td>9.28</td>
<td>2,4,6,10,11,12,14,15,21,23,24</td>
<td>11</td>
</tr>
<tr>
<td>6-chloro-2,4-dinitroaniline</td>
<td>215.9813</td>
<td>215.9812</td>
<td>-0.46</td>
<td>C6H4ClN4O4</td>
<td>185.9828 – 155.9848</td>
<td>8.25</td>
<td>1,2,3,4,5,6,8,10,11,12,15,20,21,22,23,24</td>
<td>16</td>
</tr>
<tr>
<td>2,4-dinitroaniline</td>
<td>182.0201</td>
<td>182.0202</td>
<td>0.55</td>
<td>C6H4N2O4</td>
<td>152.0218 – 122.0236</td>
<td>6.95</td>
<td>1,2,3,4,5,6,10,11,12,14,15,20,21,22,23,24</td>
<td>16</td>
</tr>
<tr>
<td>2-chloro-4-nitroaniline</td>
<td>170.9959</td>
<td>170.9961</td>
<td>1.17</td>
<td>C6H4ClN2O2</td>
<td>135.0190 – 140.9978</td>
<td>7.66</td>
<td>4,6,11,12,13,14,15,23,24</td>
<td>9</td>
</tr>
<tr>
<td>Name</td>
<td>Measure d m/z</td>
<td>Theoretic m/z</td>
<td>Mass accuracy (ppm)</td>
<td>Formula</td>
<td>Fragmentation pattern (m/z)</td>
<td>RT, (min)</td>
<td>Confirmed in sample n°</td>
<td>Detection frequency</td>
</tr>
<tr>
<td>-----------------------</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triphenyl phosphate</td>
<td>327.0777</td>
<td>327.0786</td>
<td>-2.75</td>
<td>C\textsubscript{18}H\textsubscript{15}O\textsubscript{4}P</td>
<td>233.0357 - 153.0697</td>
<td>12.4</td>
<td>11,17,20,22,24</td>
<td>5</td>
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<tr>
<td>Tributyl phosphate</td>
<td>267.1717</td>
<td>267.1725</td>
<td>-3.03</td>
<td>C\textsubscript{12}H\textsubscript{27}O\textsubscript{4}P</td>
<td>98.9844 - 140.0106</td>
<td>12.3</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Acridine</td>
<td>180.0808</td>
<td>180.0813</td>
<td>-2.94</td>
<td>C\textsubscript{13}H\textsubscript{9}N</td>
<td>152.0618</td>
<td>8.9</td>
<td>3,12,13,14,24</td>
<td>5</td>
</tr>
<tr>
<td><strong>Negative ion mode</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>183.0039</td>
<td>183.0041</td>
<td>-0.71</td>
<td>C\textsubscript{6}H\textsubscript{4}N\textsubscript{2}O\textsubscript{5}</td>
<td>95.0140 - 123.0079</td>
<td>3.1</td>
<td>4,15,22</td>
<td>3</td>
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<tr>
<td>4-Nitrophenol</td>
<td>138.0188</td>
<td>138.0191</td>
<td>-1.74</td>
<td>C\textsubscript{6}H\textsubscript{5}NO\textsubscript{3}</td>
<td>108.0211 - 95.0140</td>
<td>4.6</td>
<td>1,12,13,14,15,20,22,23,24</td>
<td>9</td>
</tr>
<tr>
<td>3-Nitrophenol</td>
<td>138.0188</td>
<td>138.0191</td>
<td>-1.74</td>
<td>C\textsubscript{6}H\textsubscript{5}NO\textsubscript{3}</td>
<td>108.0211 - 80.0268</td>
<td>5.8</td>
<td>4,6,7,8,15</td>
<td>5</td>
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<tr>
<td>x-Chloro-y-nitrophenol</td>
<td>171.9798</td>
<td>171.9801</td>
<td>-1.22</td>
<td>C\textsubscript{6}H\textsubscript{5}ClNO\textsubscript{3}</td>
<td>68.9983 - 141.9819</td>
<td>3.2</td>
<td>12,13,15</td>
<td>3</td>
</tr>
</tbody>
</table>
Conclusions

Sample cleanup for the determination of nitroanilines and quinolines were developed for textile sample analysis in Paper I. Using Carbograph® 1, clean extracts were obtained and the method was applied to determine concentrations of these analytes in solvent extracts of textile materials.

In Paper II permeation through and absorption in the skin was demonstrated for the common textile contaminant benzothiazole through in-vitro experiments. A human skin mimicking membrane was used in Franz cells and flow-through diffusion cells. The permeated percentage was used in a simplified model to estimate the dermal exposure. This was further used to roughly estimate the carcinogenic and non-carcinogenic risks associated to hypothetical worn tight t-shirt.

In Paper III a method using Carbograph® 1 was developed to concentrate textile related compounds (benzothiazoles, benzotriazoles, quinolines, and nitroanilines) from water samples. In a pilot study it was used to investigate three WWTPs effluents in Stockholm, Sweden, where 1-H-benzotriazole, 4/5-tolyltriazole and UV-P were detected and quantified in the analyzed samples.

Considering the possible human exposure and environmental release, a strategy towards suspect and non-target screening was proposed in Paper IV. The non-target screening approach was able to suggest compounds belonging to nitrophenols, organophosphates, phthalates and acridines. The suspect screening approach also confirmed the occurrences of benzotriazoles, benzothiazoles, nitroanilines and quinolines in textile samples.
Future perspectives

Methods for skin permeation studies of textile chemicals should be further
developed and applied to textile related chemicals with different chemical
properties. Such studies are essential to estimate the exposure and to make
possible the assessment of associated risks for textile related compounds.
Sweat surrogate should be applied as a clothing extracting medium. Such
method could be used to experimentally calculate the migration factor of
textile contaminants using the dermal exposure model.

Target screening methods should be developed for more compound groups
that have been detected in clothing textiles. Application of these methods
could be used to suggest and prioritize toxicological studies for
compounds detected at high concentration levels in various textiles. Also,
the number of investigated clothing textiles should be increased, and the
methods applied to other textile materials.

The SPE method developed to concentrate the four target compound
groups, benzothiazoles, benzotriazoles, nitroanilines and quinolines, from
aqueous samples should be modified in order to investigate the emissions
from laundring of textile materials.

Non-target and suspect strategies should be further developed and applied
to both a laundry wastewater and wastewater treatment plant effluents to
identify compounds which are released during the laundring and released
to but not removed during wastewater treatment. This would also be a way
to measure wastewater treatment plants´ efficiency in removing chemicals
from the effluent stream.
Acknowledgments

To begin with, I would like to thank the department of Environmental Science and Analytical Chemistry (ACES), in particular the Analytical Chemistry unit (ACES-k), to give me the possibility of being a PhD student and the financial support. I also want to thank the Lipidor for co-founding and the great support they offered during my studies.

I couldn´t achieve this result without the guide and the inputs offered by my supervisors Conny Östman, Carlo Crescenzi, and last but not least Jan Holmbäck. Thank you to accept me as PhD student, for your guidance, support and as well as to give me the opportunities to be involved in interesting projects. I want express my gratitude to Jan to have been always present and ready to help me in any circumstances (Thank you Jan!), and as well as to Conny that always supported my ideas (Thank you Conny!). A special mention goes to Carlo, you always treated me as a member of your family. I vividly remember the first time when you sent me in Sweden. You gave to me the keys, detailed instructions to reach the apartment and some Swedish moneys, as one would do for his own son (Thank you Carlo!).

During my studies it was a pleasure to collaborate with all my co-authors (in alphabetic order) Ahmad Amini, Anders Colmsjö, Annika Jahnke, Carlo Crescenzi, Conny Östman, Damien Johann Bolinius, Emanuele Moccia, Emilia Strzalka, Giovanna Luongo, Hirsh Koyi, Jan Holmbäck, Jonas Eklund, Josefine Carlsson, Matthew MacLeod, Michele Dario Manniello, Mohamed Abdel-Rehim, Paola Russo, Rozanna Avagyan. I am grateful for the scientific discussions, meetings and the revisions we went through together.
Huge thanks goes to the present staff at unit of Analytical Chemistry (alphabetic order): **Alessandro** Quaranta, some of my best memories are with you (Ti Vi Bi, Ti Ti Tì); **Amina** Souihi.; **Anneli** Kruve, thank you for the scientific discussions and the comments received during the internal review of this thesis, I really appreciated your dedication; **Cynthia** de Wit, thank you for the support you gave to all PhD students, included me, while you were the unit head; **Emila** Eklund, thank for dedication in your diploma projects and later as lab assistant; **Hatem** Elmongy, man it was a pleasure to meet you; **Isabella** Karlsson, it was a pleasure to meet you at department, you always treated me as friend (Thank you!); **Josefine** Carlsson, besides to be a co-author, you also have been a great office mate and friend (Thank you!); **Lena** Elfver, when I was sad because the instruments were not working, I always would have find your big smile, this was very important (Thank you!); **Leopold** Ilag; **Lorena** Ndreu, it was a pleasure to meet you at department, I will never forget the time we spent together (Thank you!); **Magnus** Åberg, it was a pleasure to share coffee and mozzarella with you; **Nikola** Randoman; **Pedro** Sousa, you were always open to explain “your stuff” to me (Thank you!); **Roger** Westerholm, it was a pleasure to meet you during my study; last but not least **Ulrika** Nilsson, you were unit head at time I was admitted as PhD student, you always believed in my abilities (Thank you!).

I want to thank also the old stuff of Analytical Chemistry: **Ahmed** Ramzy, your support was very important for me (Thank you!); **Aljona** Seleh; **Aziza** El Beqqali; **Chrisatoffer** Bergvall; **Erik** Tengstrand; **Farshid** Mashayekhy rad, you took care of everyone and you were always ready to help, I wish everyone could find a special person as you in their life (Thank you!); **Giovanna** Luongo; **Gunnar** Thorsen, I want to thank you for dedication and effort you put in giving always constructive inputs; **Ingrid**
Granelli; Ioannis Sadiktsis, we shared good time together; Javier Zurita, it was a pleasure to meet you in the department, you are a great friend (Thank you!); Jonas Fyrestam, it was a pleasure to share the office and my time with you, you made me feeling at home all the time (Thank you!); Jonas Rutberg, you have been always kind with me (Thank you!); Karin Englund, although I was always loosing lab staff, all the time you were kind with me (Thank you!); Liyng Jiang, you were very kind to me, thank you for the support you gave me when I was lab assistant in bioanalytical course; Mohamed Abdel-Rehim, it was a pleasure to be your students (Thank you!); Mohammad Moein; Nadia Zguna; Nicoló Riboni, I discover that polenta it is actually good, we shared good time together (Thank you!); Petter Englund, You are the best Swedish I met (Thank you!); Rozanna Avagyan, it was a pleasure to share the office with you (Thank you!), Rudolf Andrýs, I could always rely in your lighter (Thank you!); Silvia Masala; Trifa Mohammed Ahmed; Vibhu Rinwa, it was a pleasure to share my time with you due to the happy presence you brought.

A special mention goes to Giovanna Luongo and Pau Beneto Valles, you have been my family in Sweden, it has been very difficult without you here (Thank you!). Hwanmi Lim, I don’t know how I would have done with instruments without your calm and kindness (Thank you!). Alessandro Quaranta, you found the time to help me every moment I needed (Thank you!). I don’t have word to express my gratitude to Pol, he is simply the person with biggest heart in the word, (Thank you Pol!).

I would like to extend my gratitude to the people of former department of Environmental Chemistry and in the other side of the corridor: Margareta Törnqqyst; Lillemor Asplund; Henrik Carlsson; Hithesh Motwani; Johan Eriksson; Jonathan Martin; Ioannis Athanassiadis, thank you to be always ready to help (Thank you!); Jenny Aasa; Jana Weiss; Hongyu
Giulia Martella and Stathis Vryonidis, I will miss our breaks (Thank you!).

I would like to thank all my students, I was lucky to meet you all during my studies: Alessia Viola; Claudia Cascone; Davide Sorricelli; Emanuele Moccia; Emilia Strzalka; Gabriel Kalli; Gergely Szabados; Jonas Eklund; Linnéa Soldemo; Malin Ekholm; Paola Savino; Roberto Gerardo Di Meo; Rosa Amati; Tim Åström.

I also I want to thank Silvana Vasile, (Ti Vi Bi, Ti Ti Tí); Giuliana Grasso; Philippe Anfray; Annamaria Iannuzzi, Debora Tommaseo, Elena Passarini, Donato Varalli, Serena De Chiara, Nesrine Mansouri, Cristiana Margarita, Federico Zacchia, Claudia, Pietro, Chiara, Enrico. I want to thank also Rickard, I could always count on you in Sweden (Thank you!), Maria Teresa and Franco.

I want to thank also my dear friends visiting me in Sweden: Jimmy and Chiara, Jerry and Emy, Aldo Padovano and Federica Amendola, Marietto Di Mieri and Graziella Migliorin, least but not last Diego Tatulli. I want to thank also to my 5 Sc. Bronx, always present over the years and all my other best friends: Luca, Vincenzo (detto pecora), Sebastiano, Demetrio; vi voglio bene.

E poi ci siete voi, Mamma (Rosanna) e Papá (Vincenzo). Vi ringrazio per aver assecondato e supportato i miei sogni ed ambizioni, sono fortunato ad avere dei genitori come voi. Un pensiero special va anche alla mia nonnina (Lina), avrei solo voluto spendere più tempo insieme a te. Un ringraziamento va anche alle mie sorelle, Grazia e Enza, alla mia amata ZiEttà, zio Mino, Rossella, Costantino e alle mie nipotine (Rosanna e Antonietta).

A questo punto credo di non aver dimenticato nessuno, ma certo che si: la mia amata Mila. Sei le fondamenta di tutti miei successi, la persona che
non mi ha mai abbandonato. Grazie per tutto quello che fai per me ogni giorno.
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