Chemicals in textiles

A potential source for human exposure and environmental pollution

Giovanna Luongo

Doctoral Thesis

Department of Environmental Science
and Analytical Chemistry

Stockholm University

2015

Front cover: Roberta reading in the garden, wearing a skirt 100% cotton, made in India (sample not included in the present study)(Foto taken by Fabio Parisi).

A mia sorella Roberta

Table of contents

Abstract

The wide use of chemicals in textile production is common knowledge, whilst very little has been done to disclose the potentially harmful compounds hiding in our closet.

The initial part of this work focused on explorative screening of textile materials in common clothing. Non-targeted analysis of a set of sixty garments revealed the presence of thousands of compounds, among which over a hundred were tentatively identified.

Depending on the frequency of occurrence in textile, skin penetrating properties and toxicological data, candidate compounds were selected for confirmation.

Analytical methods were developed for their identification and quantification, with focus set on four groups of compounds: quinolines, benzothiazoles, benzotriazoles and aromatic amines. The analytical methods are based on ultrasonic-assisted extraction, followed by solid phase clean-up, combined with GC/MS or HPLC/MS/MS analysis. Concentrations of many target analytes were notably higher in polyester samples compared to garments made from cotton and blended material.

The release during washing was investigated for two of the compounds groups, quinolines and benzothiazoles. The decreased concentrations in the garments suggest that laundry is a source of emission of these chemicals into household wastewater, and possibly further into the aquatic environment. Due to the slow decrease of the concentration in the garments when washed, substantial amounts of the compounds will remain in the textiles for a long time, with the possibility of exposure to the skin of potential harmful compounds as a result.

Populärvetenskaplig sammanfattning

Stora mängder vatten och ett stort antal olika kemiska föreningar är involverade i de olika produktionsstegen i textiltillverkningsindustrin.

Många av de föreningar som inte är bundna till textilfibrerna tvättas ur under tillverkningen och hamnar i avloppssystemen, medan andra blir kvar i den slutliga produkten. I kontakt med huden kan dessa utgöra ett hot mot människors hälsa då flera av dessa kemikalier har visats vara hudirriterande, allergi- och cancerframkallande och reproduktionstoxiska.

Europeiska unionen har antagit REACH förordningen, med det huvudsakliga målet att skydda människors hälsa och miljön från risker från användandet av olika kemikalier. Förordningen innebär att industrin måste registrera de kemikalier som importeras eller produceras inom EU och överstiger en mängd av 1 ton. Industrin ansvarar även för att bedöma och hantera riskerna med kemikalier och tillhandahålla lämplig säkerhetsinformation till sina användare. EU kan vidta ytterligare åtgärder om ämnen har särskilt farliga egenskaper och implementeringar i REACH-lagstiftningen planeras i flera steg fram till 2018.

Det är dock svårt att göra en omfattande karakterisering av de organiska föroreningar som finns i tyger och textilier, med tillhörande miljö- och hälsopåverkan. Detta på grund av antalet mellanhänder från produktion till färdig produkt, snabba förändringar i användandet av kemikalier och snabba förändringar i modet som styr tillverkningen av textilier och kläder. För många av de nya kemikalierna, i synnerhet färgämnen, saknas det dessutom miljö- och hälsodata.

En screeningmetod av kemikalier i textilier, med särskild tonvikt på kläder har tagits fram i detta arbete. Över hundra kemiska föreningar identifierades preliminärt och förekomsten av mer än fyrtio föreningar säkerställdes i en uppsättning av sextio kläder som säljs/såldes på den globala marknaden.

Risskerna förknippade med de funna ämnena var främst hudsensibilisering och irritation, men också reproduktionstoxicitet och bevisad/misstänkt karcinogenicitet. Sju av de preliminärt identifierade föreningarna fanns med i kandidatlistan (SVHC) i REACH-förordningen.

Specifika analysmetoder utvecklades för fyra grupper av föreningar: kinoliner, bensotiasoler, bensotriasoler och aromatiska aminer.

Man kunde se koncentrationsskillnader för några av analyter som kunde relateras till textilmaterialet, där t.ex. större mängder kinoliner och bensotiasoler uppmättes i polyesterbaserade material jämfört med andra textilmaterial. När kläderna tvättades minskade mängden av dessa två ämnesgrupper i textilierna. Det tyder på att kläder kan vara en betydande emissionkälla för dessa föreningar till hushållsspillvatten och så småningom i omgivande vattenmiljöer. Urtvättningen ur kläderna var långsam vilket betyder att kemikalierna finns kvar i kläderna och är en möjlig källa till hudexponering.

List of papers

This thesis is based on the following publications, which are referred to in the text by the corresponding Roman numerals.

I. Non-targeted screening and identification of chemicals in clothing textiles
G. Luongo, G. Torshén, C. Östman
[In Manuscript]

The author was responsible for the experimental work, data evaluation and major part of the writing.

II. Quinolines in clothing textiles—a source of human exposure and wastewater pollution?
G. Luongo, G. Thorsén, and C. Östman
Analytical and Bioanalytical Chemistry (2014) 406: 2747-2756.

The author was responsible for all of the experimental work, data evaluation and major parts of the writing.

III. Benzothiazole, benzotriazole, and their derivates in clothing textiles - a potential source of environmental pollutants and human exposure
R. Avagyan, **G. Luongo**, G. Thorsén, C. Östman
Environmental Science and Pollution Research (2015)22:5842-5849

The author was responsible for parts of the data analysis and part of the writing.

IV. The washout effect of benzothiazole, benzotriazole, quinoline and their derivatives from clothing textiles
G. Luongo, R. Avagyan, R. Hongyu, C. Östman
Accepted for publication in *Environmental Science and Pollution Research*

The author was responsible for part of the experimental work, significant parts of the data analysis and significant parts of the writing. The first authorship was shared with R. Avagyan.

V. Aromatic amines in clothing textiles
G. Luongo, F. Iadaresta, E. Moccia, C. Crescenzi, C. Östman
[In Manuscript]

The author was responsible for part of the experimental work, significant parts of the data analysis and significant parts of the writing.

The following papers where published by the author but not included in the thesis.

Organophosphate and phthalate esters in Standard Reference Material 2585 Organic Contaminants in House Dust
C. Bergh, **G. Luongo**, S. Wise, C. Östman
Analytical and Bioanalytical Chemistry (2012) 402:51-59

Organophosphate and phthalate esters in settled dust from apartment buildings in Stockholm
G. **Luongo** and C. Östman
Indoor air (2015) doi: 10.1111/ina.12217

Abbreviations

1-M-iso-Q	1-Methyl-isoquinoline
2,4-DNA	2,4-Dinitroaniline
2,6-DiCl-4-NA	2,6-Dichloro-4-Nitroaniline
2-Br-4,6-DNA	2-Bromo-4,6-Dinitroaniline
2-Cl-4-NA	2-Chloro-4-Nitroaniline
2-Cl-5-NA	2-Chloro-5-Nitroaniline
5-Cl-2-NA	5-Chloro-2-Nitroaniline
6-Cl-2,4-DNA	6-Chloro-2,4-Dinitroaniline
ACN	Acetonitrile
AMDIS	Automated Mass Spectral Deconvolution and Identification System
BT	Benzothiazole
BTri	Benzotriazole
BUVS	Benzotriazole Ultraviolet Stabilizer
CT	Cotton
DCM	Dichloromethane
DMQ	Dimethyl Quinoline
EC	End Capped
EI	Electron Ionization
EPA	Environmental Protection Agency
ESI	Electrospray Ionization
EU	European Union
GC	Gas Chromatography/Gas Chromatograph
GCB	Graphitized Carbon Black
HPLC	High Pressure Liquid Chromatography
Iso-Q	Isoquinoline
K_{ow}	Octanol-water partition coefficient
LC	Liquid Chromatography/Liquid Chromatograph
LLE	Liquid Liquid Extraction
LOD	Limit Of Detection
LOQ	Limit Of Quantification
MBT	2-Mercaptobenzothiazole
MeOH	Methanol

m-NA	m-Nitroaniline
MQ	Methyl Quinoline
MS	Mass Spectrometry/Mass Spectrometer
MS/MS	Tandem Mass Spectrometry
MTBT	2-Methylthio-Benzothiazole
NIST	National Institute of Standards and Technology
o-NA	o-Nitroaniline
PA	Polyamide
PCA	Principal Component Analysis
p-Cl-A	p-Chloroaniline
PE	Polyester
p-NA	p-Nitroaniline
PREG	Polar Retention Effect on Graphite
Q, q	Quadrupole
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RSD	Relative Standard Deviation
SIM	Selected Ion Monitoring
SPE	Solid Phase Extraction
SRM	Selected Reaction Monitoring
SVHC	Substance of Very High Concern
TFA	Trifluoracetic Acid
Ttri	Tolylbenzotriazole
USE	Ultrasonic-assisted Extraction
UV	Ultraviolet
UV-234	2-(2H-Benzotriazol-2-yl)-4,6-bis(1-methyl-1-phenylethyl)phenol
UV-328	2-(2H-Benzotriazol-2-yl)-4,6-di-tert-pentylphenol
UV-P	2-(2H-5-Methylphenyl)benzotriazole

1. Introduction

Production of textiles consists of a long multistep chain involving a large number of chemically heterogeneous compounds. It is also one of the greatest consumers of water per kg of produced material. It significantly contributes to environmental pollution by discharging wastewater rich in hazardous chemicals, such as azo-dyes, flame retardants, formaldehyde, dioxins, biocides and heavy metals, which in different ways pose threats to public health. Chemicals, harmless or dangerous, can accidentally or purposely enter and leave the textile mill during different steps of the manufacturing process. They can end up in goods placed on the market, either intentionally to give specific characteristics to the article (color, softness, flame and crease resistance, or water repellent properties), or unintentionally as residual materials from the production (traces of toxic and carcinogenic compounds can often be found in commercial dyes).

Several textile operations lack quality-control systems for contaminants present in the raw fiber, left in the finished product, handled during the manufacturing processes or formed due to the use of high temperature, alkaline conditions, powerful oxidizing agents, etc. Significant amounts of hazardous compounds have been found in wastewater effluents from textile production plants [1-3].

Moreover, clothes are worn in close contact to the skin and, if chemicals are present in the garments, wearing them is a possible route for human exposure.

Increasing environmental awareness has recently pushed an enhancement of international standards and regulations concerning quality, safety and sustainability of the textile industry. Since 2006 the European Union (EU) has adopted the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation to protect human health

and the environment from risks posed by chemicals [4]. EU introduced restrictions, applied to any compound on its own, in a mixture or in an article, which limit or ban manufacture, placing on the market and/ or use of a substance [4]. Implementations of REACH legislation are planned in several steps until 2018.

Nevertheless, a comprehensive characterization of organic contaminants present in fabrics and the related environmental impact are hindered due to the large number of intermediaries involved in the production steps, and to the extensive and rapid change in the chemicals usage, caused by fashion trends. For many of the newly introduced substances, especially dyes, environmental and health impact data are scarce. Furthermore, many of the used compounds might not fall under the obligation for registration, since their consumption does not exceed one metric ton. However, if they have low chemical and biological degradation rates, they might accumulate in biological tissues and their concentrations magnified in organisms exposed to contaminated environments.

This points out the strong need for developing analytical methods to effectively detect and control pollutants discarded into the environment as well as hazardous compounds, which consumers could be exposed to during the use.

2. Textile chain

2.1 Fibers

The textile chain starts with the production of raw fibers, which can be classified into two general categories:

Natural: e.g. *cotton, wool, silk*

Artificial: e.g. *petrochemical origin, regenerated cellulose*

Pesticides and herbicides might be used during the cultivation of cotton, as well as biocides and fungicides during its transportation and storage, while parasiticides are commonly applied on sheep to control external parasites [5, 6]. Even though their concentrations in the final products are too low to be detected, these contaminants can be present in the raw material and released from it during heating or scouring processes on the fiber; hence, moving these into the aquatic environment, potentially create pollution problems.

Pentachlorophenol, an organochlorine pesticide, also used as fungicide during transportation, has been detected in textiles [7].

Synthetic fibers may also contain very broad categories of impurities; e.g. solvents (when raw material undergoes solvent-spinning processes to make the fiber), by-products of polymerization processes (monomers, oligomers, catalysts, and solvents), and additives (antistatics, lubricants) [8].

Alcohols (C_{14}-C_{22}), esters of carboxylic acids (C_{14}-C_{24}), hydrocarbons (C_{14}-C_{18}), carboxylic acids (C_{16}-C_{24}), and phthalate esters have been detected in wastewaters from synthetic fiber dyeing and finishing operations, as well as in extracts from synthetic fibers such as polyester, acrylic and nylon-6 [8].

2.2 Production process

The textile production processes are schematically presented in **Figure 2.1**.
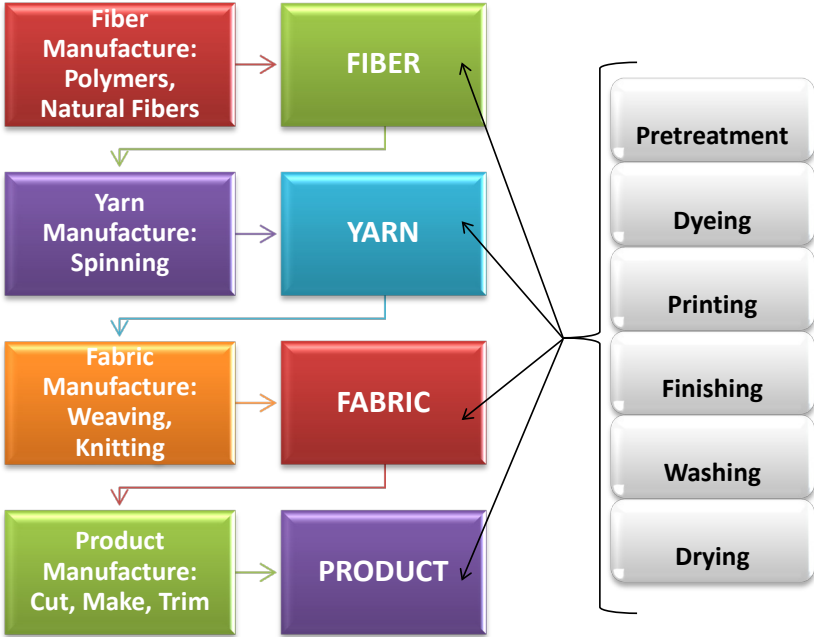

Figure 2.1 Textile processing flow-chart

Pretreatment processes (desizing, scouring, bleaching, and mercerizing) are performed on the fiber in order to remove undesired material and improve the affinity for dyeing, printing and finishing treatments [9]. Each production step, briefly described below, implies the usage and the discharge of vast array of chemicals [10].

Desizing is a process used to remove size material from textile [11]. It can represent an environmental problem when the compounds used, which might be found in effluents from the processing plants, are difficult to emulsify or hardly biodegradable, like silicon oils [12].

Hydrophobic substances, natural or added, such as oils, waxes, gums, which do not absorb the dyes or which are dyed with different color, result in considerably impaired dyeing. These compounds have to be removed before the dyeing, by a process of *Scouring* [12]. This process uses compounds such as anionic or non-ionic surfactants (e.g. alcohol ethoxylate, and alkyl phenol ethoxylate), dispersing and reducing agents or chelating agents for removing metal ions [12].

Bleaching is a chemical process to remove unwanted, extraneous substances in order to improve the whiteness of the textile [13]. The properties of bleaching agents depend on the type of fiber and often a combined bleaching treatment is needed. The most frequently used compounds are hypochlorite or chlorite and hydrogen peroxide together with many auxiliary compounds such as stabilizer, co-stabilizer, wetting agents, activators, and anti-corrosion agents [14].

Mercerizing is a chemical procedure that improves the dyeability and increases the strength, dimension stability and luster. Several compounds like alcohol sulphates, anionic surfactants, and cyclohexanol can be used [15].

Dyeing is the process to color textile material. This operation can be done during various stages (on the fiber, yarn, fabric and product) alone or together with other treatments [16]. It involves preparation of the dye, dyeing, fixation, washing, and drying.

Dyes contain chromophoric (usually double-bonds, aromatic and heteroaromatic rings, responsible for coloring properties) and auxochromic groups (groups forming salts, responsible for dyeing properties) [17]. With regard to the structure and usage, dyes are classified into azo-dyes, anthraquinone dyes, acidic (for wool, nylon, silk) or basic, disperse dyes (used for polyester and other synthetics material), reactive and direct dyes (used for cotton and viscose), and metal complex dyes (generally chromium or cobalt complexes) [18].

The issue of carcinogenicity related to azo-dyes has already been addressed [19]. Mainly used for yellow, orange, and red coloring, they represent the majority of the synthetic dyestuffs. The structure contains one or more azo-bonds, which can be cleaved, releasing potentially carcinogenic aromatic amines [20, 21].

After dyeing treatment, a large amount of the non-fixed dye leaves the dyeing units together with other auxiliary substances added during the process, resulting in a contaminated effluent water stream, which should be treated to remove the chemicals before being released into the environment. Several of these compounds are non-biodegradable and are released non-transformed into the wastewater system [22].

Wastewater released after the dyeing process might contain, beside dyes, dyeing additives like ethoxylates, alkylphenol ethoxylates, retarders for cationic dyes, dispersing agents, ethylenediamine tetraacetate and many others [23].

Printing is the process where pigments, which are generally insoluble, are applied on the textile to give specific patterns, e.g. text on the garment. Possible pollutants from printing are: dyes or pigments, and organic solvents.

Finishing encloses all those treatments, mechanical and chemical, performed on fiber, yarn, or fabric, in order to improve look and texture [24]. Possible pollutants released due to finishing processes are: formaldehyde, resins, flame-retardants, antistatics, softenings, cross-linking agents and biocides [25].

3.   Release of chemicals from textile usage

Effluents of textile-dyeing plants are extremely difficult to treat due to their high content of pollutants such as polycyclic aromatic hydrocarbons, heavy metal ions, surfactants, dyes, solvents, detergents and recalcitrant compounds [26-28]. Dyes and other contaminants [29], octyl- and nonylphenol, their ethoxylates and carboxylates derivates have been detected in textile wastewater treatment plants, as well as in stream effluents and surface waters [30-32].

Bioassay toxicity tests made on the chemicals released to water from various textile plants [33], together with studies on the modifications of the habitat [34], revealed a need for implementation and enforcement of the strategy for monitoring and protecting the streams.

However if the rather heavy release of chemicals during textile production is a well-known environmental problem, what about the finished product?

Several studies on chemicals in textile have been made during the years. They include biocides [35], hexabromocyclododecane flame-retardants [36], organophosphorous pesticides [37], per- and polyfluoroalkyl substances [38-40], polycyclic aromatic hydrocarbons [41, 42], formaldehyde [43], nonylphenol ethoxylate and the release through laundering [44], azo-dyes and their reduction products [45, 46], polychlorinated dibenzo-p-dioxins, dibenzofurans and octachlorodibenzofuran [47, 48].

Dioxins have been shown to be present in dyes and to magnify their concentration in textile after the dyeing processes [49]; they were also formed during textile processing and incineration [32, 50, 51].

Hazardous substances, which remain in the fabric, may, via diffusive transport, be transferred to the *stratum corneum* of the skin, leading to

dermal absorption and systemic exposure. Textile clothes, bed linen and towels are articles of every-day use, which come in close contact with the human skin.

The skin is the largest human organ, which has a vast area exposed to the environment. Molecules with a weight less than 1000 Da and a log K_{OW} between 0.7 and 5.9 have the potential to cross the epidermal barrier, with maximum absorption between 1 and 2 [52-54].

Experiments to show transfer of organic compounds from contaminated textile to the outermost layers of the skin have been made. Klasmeier et al. [55] have demonstrated the transfer to skin from different cotton T-shirts containing high levels of hepta- and octachlorinated dibenzo-*p*-dioxins and dibenzofurans.

In a scientific study by Gallagher et al. [56], volunteers were made to wear shorts and T-shirts/tank tops during 5 minutes of mild exercise, which made them sweat; subsequent analysis of the skin revealed, for some of the tested persons, the presence of benzothiazole on the back (covered by textile) but not on the forearms (not covered by textile), indicating the release of chemicals from clothes. A study proving the transfer of polychlorinated dibenzo-p-dioxins from textile to the skin has also been made [55]; however, systemic exposure is only possible if the compounds are able to overcome the penetration barrier posed by the viable epidermis. An *in vivo* experiment performed by Blum et al. in 1978 [57] demonstrated that a chemical present in clothes can enter the human body via dermal absorption. In this study pajamas containing 5% tri (2,3-dibromopropyl) phosphate (a flame retardant, Mw = 697.6 Da, Log K_{OW} = 3.71), was worn by children during sleep (8 hours exposure period). The following morning a fifty-fold increase in the metabolite 2, 3-dibromopropanol was detected in the urine. After changing to a pajama free of the substance, the urine concentration of the metabolite slowly decreased, but, after five days, it was still twenty times higher compared to the initial concentration.

So far, the risks shown to be related to textile exposure are mainly dermatitis caused by azo-dyes [58, 59]. However, a large number of chemicals found in textiles (perfluorinated compounds (PFCs), organophosphorous pesticides, polycyclic aromatic hydrocarbons (PAHs), dioxins, nonylphenol ethoxylate and phthalates) can pose more severe risks to human health like cancer, immunotoxicity, as well as reproduction and development hazards [60-65].

Even if exposure to chemicals from textiles is supposed to take place through skin contact, other up-take routes cannot be ignored. By evaporation, substances can migrate into the air, or due to wear, fibers can be released to the air giving additional routes of human exposure through inhalation or ingestion. Not only clothes, but several other textile articles are present in the indoor environment and can all summed up to entail an exposure source of potentially high importance.

The picture of textiles as a source of chemical pollution can be broadened if we consider the washing of textiles. Compounds in the fabric can be washed-out during laundry and, via wastewater and sewage plants, reach the aquatic environment. The pattern for chemical release from textiles, presented in **Figure 3.1**, shows how textiles contribute to environmental pollution.
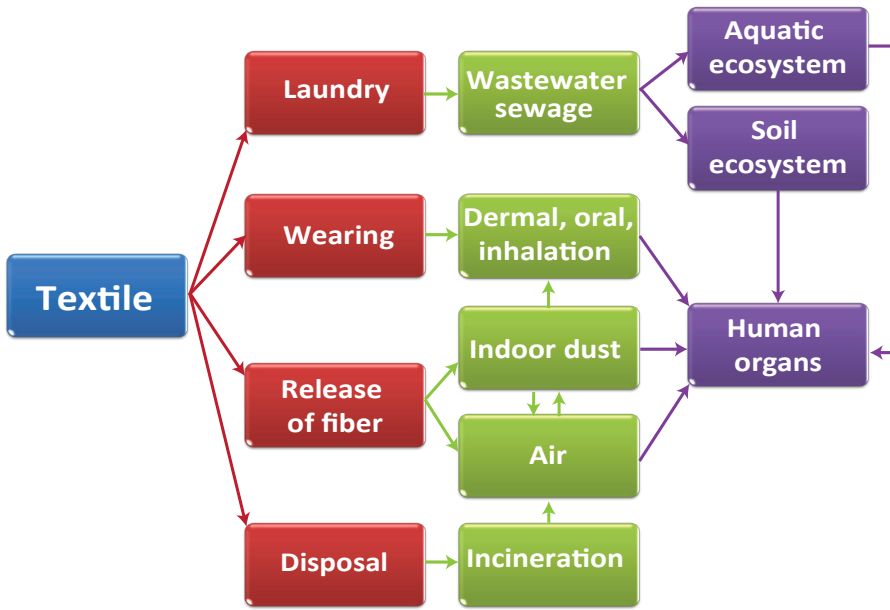
Figure 3.1 Chemicals release pattern for textiles

3.1 Quinolines (Paper II and IV)

Quinoline is an organic base belonging to the group of azaarenes, heterocyclic aromatic compounds with one or more nitrogen atoms placed in the aromatic ring, **Figure 3.2**.
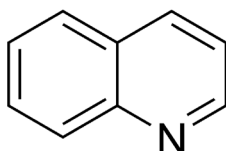

Figure 3.2 Quinoline structure

The principal sources of quinoline are coal tar and petroleum, and the main uses as a chemical intermediate, corrosion inhibitor and as a solvent for resins and terpenes [66, 67], may result in its release to the environment through various wastewater streams. Its industrial application related to textiles is the manufacture of dyes, such as 2- and 4-methyl derivatives, that are precursors to cyanine dyes [68].

Like pyridine derivatives and other azaarenes, quinoline is often reported as an environmental contaminant. Its presence has been associated with effluents from processing shale oil or coal facilities [69], and it has been found in urban air particulate matter and tobacco smoke [70-72]. Because of its relatively high solubility in water (quinoline is slightly soluble in cold water but dissolves easily in hot water), it has significant potential to cause water contamination [73]. Its presence has been shown in ground water, lake and marine sediments [74-77], and quite recently in Dutch river sediments and in the coastal zone of the North Sea [78, 79], as well as in dye processing plants effluents [80, 81].

Due to its log octanol/water partition coefficient (log K_{OW}) of 2.03, quinoline has potential to be absorbed through skin. It has been shown to cause skin irritation and chromosome aberrations in rat liver [82]. Even though no carcinogenicity studies on humans are available, it has

been shown that quinoline and some of its methylated isomers induce hepatocellular carcinosarcoma in mice [83, 84], and EPA (Environmental Protection Agency) has classified it as a Group B2 chemical, a probable human carcinogen [85].

3.2 Benzothiazoles and Benzotriazoles (Paper III and IV)

Benzothiazole (BT) and benzotriazole (BTri) are aromatic heterocyclic compounds where benzene is fused with a five member ring, which contains sulfur and/or nitrogen as heteroatoms, **Figure 3.3**.
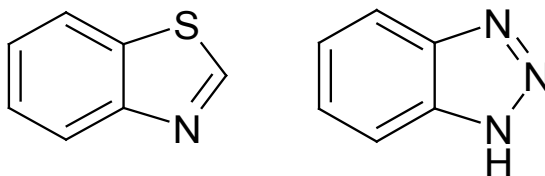

Figure 3.3 Benzothiazole (left), Benzotriazole (right) structures

Benzothiazole, mostly as derivative with the 2-position of the thiazole ring being substituted, finds its application in rubber production as vulcanization accelerators [86], and in other industry applications as biocides [87]. These compounds are also commonly used as corrosion inhibitors [88], while 2-hydroxyphenyl derivatives of benzotriazoles are used in textiles as Benzotriazole UV stabilizers (BUVS) [89, 90].

BTs and BTris are rather water-soluble, but not readily degradable, thus, they are not completely broken down in wastewater treatment plants and a significant part reaches surface water [91, 92].

BT occurrence is widespread; it has been found in municipal and household wastewater [92, 93], whilst BUVSs have been found in fish [89], clams, oysters, and gastropods [90]. BTri are released from automobile tires as well, and 2-Mercaptobenzothiazole (2-MBT) and BT are present in urban air, most likely due to tire tread abrasion [94].

Their acute toxicity in various test systems has been shown [95] and also allergenic and irritating properties [96, 97]. Benzotriazole was found to be mutagenic and estrogenic in aquatic organisms [98] and BUVSs can bioaccumulate in birds, fishes and invertebrates [90]. Due to the octanol-water partition coefficient, they can be absorbed by the skin. Transfer to skin experiment showed that benzothiazoles are released from textile clothes [56], moreover both classes of compounds have been detected in urine samples collected from general populations [99].

3.3 Aromatic amines (Paper V)

Aromatic amines have one or more aromatic rings connected to one or more amino groups; the simplest is represented by aniline, **Figure 3.4**.
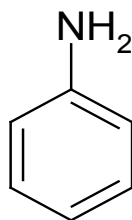

Figure 3.4 Aniline structure

Depending on the number of aromatic rings or/and the substituents bonded to the aromatic ring, they have different environmental and health related impacts. Sources can be oil refining processes, diesel engine exhaust, synthetic polymers, textile and hair dyes, rubber, pesticides, and pharmaceuticals [100].

Their use related to textile industry is chiefly in the production of azo-dyes, whose chemical structure is more complex than that of the parent amines [101]. However, when the dye is absorbed and metabolized in the body, the cleavage of the vulnerable azo-bond releases the amine, causing high risks to human health [20, 102]. Certain dyes have a low degree of percutaneous absorption, nevertheless, bacteria of the human

skin have been demonstrated to metabolize azo-dyes in to aromatic amines which are known to easily penetrate the skin [103].

Studies on workers exposed to 2-naphthylamine, benzidine, and 4-aminobiphenyl showed association between human exposure to aromatic amines with an increased risk of urinary bladder cancer [104, 105]. O-toluidine has also been suggested to be a bladder carcinogen. Workers exposed jointly to o-toluidine and 4,4'-methylene bis(2-methylaniline) showed 62-fold increase in bladder cancer risk [106].

4. Chemical analysis of textiles

4.1   Samples

Textiles analyzed in this thesis work were purchased between 2010 and 2014 from several stores common in the Swedish market, mainly representing European as well as worldwide brands. **Figure 4.1** indicates information regarding manufacturing countries and the materials of the samples.
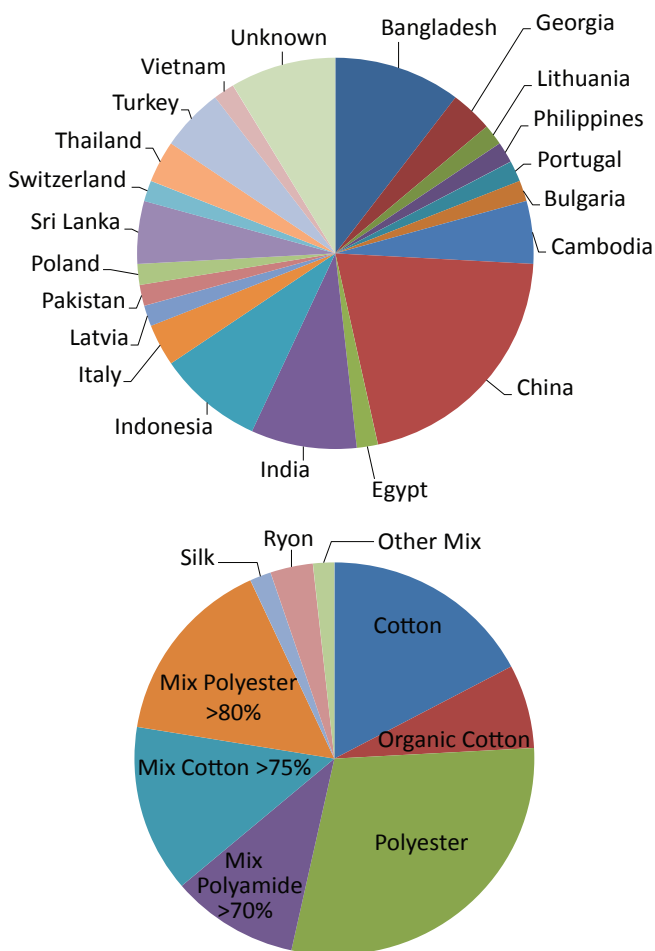

Figure 4.1a Manufacturing countries (top) and materials (bottom)

The origin of textiles manufacture was 80% Asian countries, 15% European, and 5% of unknown origin. The work focus is on textiles made from different materials, worn in contact with the skin, for which reason the samples are mainly clothes. In the chemical analysis, prints, coatings and laminated films on textile were excluded. The scope did not include garments made of technical fibers (like GORE-TEX®), non-textile fiber elements, or other details of different material on the textiles.

4.2   Extraction and clean-up

4.2.1 Ultrasonic-assisted extraction

Ultrasonic-assisted extraction (USE) was the method of choice used in the present thesis work, given the several advantages that it has compared to other techniques. It is fast, requires low amounts of solvent, and samples can be run in parallel.

The samples, cut into pieces of 5 x 5 mm, were immersed in organic solvent and placed in an ultrasonic bath at 30˚C. The system generates ultrasonic waves with frequencies above 20 kHz traveling through the medium. The expansion and compression cycles of the waves produce hot-spots in the liquid, assisting the extraction process [107].

Efficiency was evaluated by exhaustive extraction of several textile samples, using hexane, dichloromethane (DCM), DCM: toluene (80:20), and acetone as organic solvents. Extraction times (10 min, 30 min, 1 hour) as well as single and repeated extractions were also examined. It was shown that two extractions of 10 min each, using DCM, were sufficient to obtain >80% yield for a number of different compounds (**Paper I**), quinolines (**Paper II**) and anilines (**Paper V**). Slight, but not statistically significant, differences (5 to 10%) were noticed depending on the textile fibers, with cotton samples giving the highest recovery.

Deuterated internal standard was spiked into the sample prior to the extraction procedure in order to compensate for losses of the target analytes during sample preparation. For BT and BTri and their derivatives, a method developed by Avagyan et al. [108] was used (**Paper III**). This method used USE with dichloromethane: acetone (4:1) and two extraction cycles of 20 min.

4.2.2 Clean-up

Extracts were often rich in fibers and pigments. Clean-up was a necessary step to remove interferences from raw extracts, which could be co-eluted from the chromatographic system or increase the noise and, thus, the limit of detection (LOD) of the method, or decrease the uptime of the analyzing system. Since the main goal of this study was to investigate as many compounds as possible present in textile, very little sample preparation was used after the extraction.

Sample preparations were tested on a number of samples for a wide range of compounds which were found to be present in the textile samples (**Paper I**).

For benzothiazoles and benzotriazoles analyses, a syringe filter was used as the single clean-up before introduction into the HPLC/MS/MS system (**Paper III**). A solid phase extraction (SPE) cartridge packed with end-capped C_{18} was used in **Paper I**, **II** and **IV**, while Carbograph material was tested for aromatic amines and quinolines in **Paper V**.

The advantages of using SPE are speed, low solvent consumption, possibility to run several samples in parallel, quantitative extractions and the use of disposable cartridges, which reduce risk of cross-contaminations.

SPE is mostly used for sample extraction, concentration, and clean-up of liquid samples. SPE phases are available in a wide variety of adsorbents and sizes, and can be used in different operation modes such

as normal phase, reversed phase and ion exchange. The sorbent can be selected to strongly retain the analytes of interest and elute interfering compounds during the washing steps, and subsequently elute the target compounds using solvents with higher elution strength, or in the opposite manner, strong retention of interferences and fast elution of the target compounds [109, 110]. The latter case was applied by using a C_{18} end-capped (EC) SPE cartridge. This phase is an octadecyl substituted silica with the residual silanol groups end-capped. It is used for reversed phase extraction of non-polar to moderately polar compounds. By using DCM sufficient elution strength was obtained to elute quinolines and other slightly polar compounds while some of the pigments were retained. The SPE also gave a filtering effect retaining fibers from the fabric. The recoveries were determined for the C_{18} (EC) phase and a number of other SPE phase types, such as Florisil™, a magnesium silicate typically operating under normal phase conditions. C_{18} (EC) gave higher recoveries and lower relative standard deviations (RSDs) compared to other phases. However, it should be noted that there was a variation in the yields occurring between the brands, and also to some extent between batches within the same brand. This may influence the recovery of more polar compounds, and is most probably due to variations in the activity of the phases, *i.e.* the efficiency of the coverage of the residual silanols. When more polyester samples with peculiar characteristics, like simile leather texture or shining surfaces, were analyzed, the clean up using a C_{18} (EC) SPE, which was sufficient for samples tested in **Paper I**, **II** and **IV**, was not satisfactory. When undesired compounds, like pigments, entered the GC system it caused contamination of the liner as well as the column, resulting in a successive increase in peak tailing. The injector end of the GC column had to be cut often, and the liner had to be changed more frequently, in order to keep good peak shape. SPE phase based on graphitized carbon black gave better results in terms of clean-up efficiency, removing a large part of the pigments present into the raw extract (**Paper V**).

4.2.3 Graphitized Carbon Black (GCB)

Graphitized sorbents were made as an alternative to conventional reverse phase, such us silica based (C-18), where it is difficult to eliminate or shield the free polar silanol groups.

They are obtained by heating carbon blacks at high temperature (2700–3000 ˚C), resulting in a high number of carbon atoms laid out in hexagonal arrangement, with sp^2 hybridization [111].

Diverse types of interactions are involved in the chromatographic behavior: hydrophobic (London dispersion force), electrostatic: polar retention effect on graphite (PREG), interactions with the planar surface, and specific interactions with impurities [112].

Since these sorbents are generally non-specific, non-porous, and characterized by homogeneity of the hydrophobic crystalline structure, London dispersion forces are the driving forces in the adsorption process. The uniform planar surface, due to the typical lamellar structure of graphite, is highly sensitive to the geometry of the molecules [113]. GCB materials show unique behavior due to the presence of various functional groups at the surface following the oxygen chemisorption. The surface framework of GCBs used in SPE was shown to contain some structures similar to hydroquinone, quinones, chromene and benzpyrylium salts [114], which are able to interact strongly with sufficiently acidic compounds [115]. Therefore, GCB has a somewhat positively charged surface that also absorbs solutes by an anion-exchange mechanism.

PREG is the theory trying to explain the retention of polar compounds: the delocalized π-electrons present on the flat surface are attracted or rejected when a compound is positively or negatively charged [116].

In studies conducted by Di Corcia et al. [117], Carbograph exhibited a higher efficiency than C_{18} for solid phase extraction of phenols, chloroanilines and organochlorine pesticides. SPE cartridges packed with Carbograph 1 (specific surface area ≈ 100 m²/g) and Carbograph 4

(specific surface area ≈ 130 m²/g) were tested for solid phase extraction of substituted anilines and quinolines. A number of solvents and mixture of solvents (Acetonitrile (ACN), ACN: Toluene 3:1, DCM, DCM: MeOH 95:5, DCM: MeOH 95:5 with trifluoracetic acid (TFA) 10 mM, DCM:Toluene 4:1) were tested on two SPE packing amounts (250 and 500 mg) of both Carbograph materials. The highest recoveries were obtained on Carbograph 1 (250mg) cartridge using DCM and DCM: Toluene (4:1) as elution solvents, **Figure 4.2**.
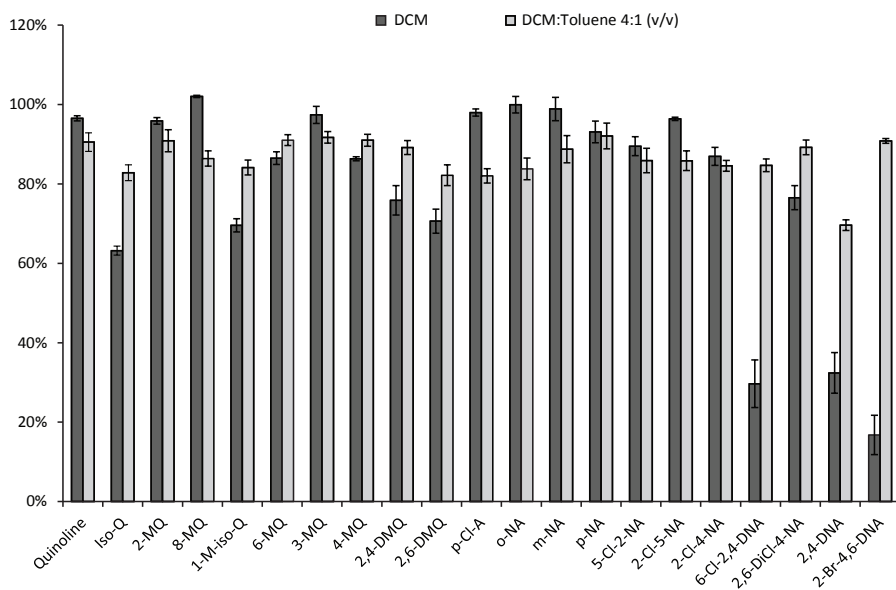

Figure 4.2: Recoveries of substituted anilines and quinolines on a 250mg Carbograph 1 SPE using DCM and DCM:Tol (4:1), (n=4)

Five fractions of 1 mL each were collected during the elution process and run on the analyzing system, showing the elution curve presented in **Figure 4.3**.
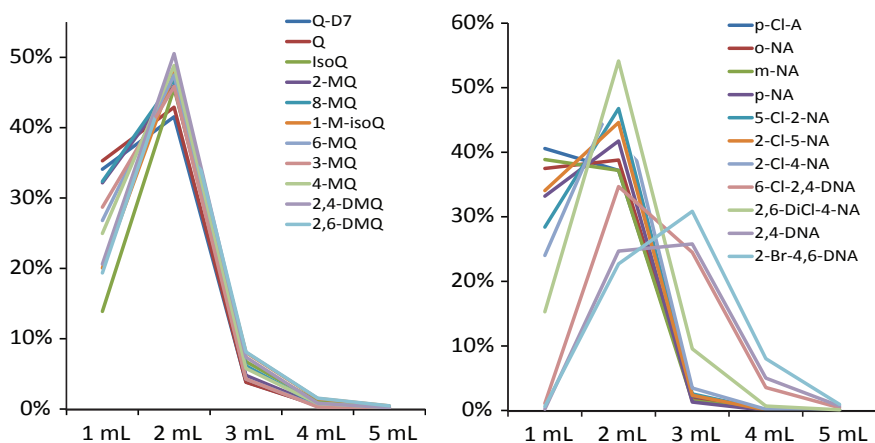
Figure 4.3: Elution curves of quinolines and anilines from 250mg Carbograph 1 SPE using 5 ml DCM:Toluene (4:1) as elution solvent

When applied to real samples, a good clean up was obtained, in most of the cases yielding SPE eluates free from pigments, **Figure 4.4**.
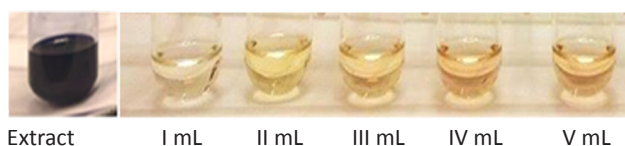

Extract   I mL   II mL   III mL   IV mL   V mL

Figure 4.4: SPE fractionation of a raw extract of a blue polyester sample. Extracted sample is the raw extract and fraction I-V is the first five ml of eluate from the SPE divided into fractions of 1mL each.

4.2.4 Liquid-liquid extraction (LLE)

Since quinolines and some substituted aromatic amine are weak bases (pKa between 4 and 6), liquid-liquid extraction was also tested as a clean-up step after DCM extraction from the fiber. DCM spiked with the target compounds was extracted three times with acidic water (pH=2). The combined water fractions were then basified with NaOH to pH=10 and the compounds back-extracted to fresh DCM. Recoveries were found to be around 70% for all the tested quinolines, chloro-

aniline and nitroanilines. However, when applied to real samples the disadvantages associated with liquid/liquid extraction, such as incomplete phase separation, gave less quantitative recoveries and an increase in the standard deviations. Further, it resulted in the disposal of large quantities of organic solvents compared to the SPE method. For these reasons LLE was not considered the method of choice for this application.

4.3 Instrumental analysis

4.3.1 HPLC/MS/MS

When using GC/MS for the analysis of benzothiazole, extensive peak tailing occurs and for some of the derivates, such as 2-Methylthio-Benzothiazole (MTBT), this effect is so strong that it cannot be analyzed. Moreover some of BT derivatives are too polar and thermolabile for GC. Thus, HPLC/MS/MS was the system of choice for the analysis of BTs and BTris. Using reversed phase HPLC with a C_8 microbore column (2.1 mm × 50 mm, 5 μm particle size), equipped with an ACE 3 C8 guard cartridge, all the target analytes could be separated, **Paper III**. Pure water and ACN with 0.10 % (v/v) formic acid were used as mobile phases. The MS parameters were determined using the direct infusion method.

4.3.2 GC/MS

For the other target analytes, as well as non-targeted screening, GC/MS was the method of choice. A number of columns with different stationary phases (DB-1, DB-5, DB-Wax, DB-17 and HP-5) were tested with respect to their ability to separate the target analytes. DB-Wax gave the best peak symmetry for the tested compounds, but due to the co-elution of some peaks and the inability to elute dinitroanilines with higher

boiling points, because of a temperature limit of 250 ˚C, this phase was not used. In spite of the tailing for some of the compounds an HP-5 MS column was used in **Paper I** and **II**, while a DB-5 MS column was used for the GC/MS analyses in **Paper IV** and **V**. Both these phases are low bleeding dimethyl (95%) diphenyl (5%) polysiloxane polymers, where the separation of the analytes depends mainly on their vapor pressure. As an example of the GC/MS separations the selected ion monitorning (SIM) chromatogram of a standard mixture of quinolines and anilines on a DB-5 MS phase is shown in **Figure 4.6**.
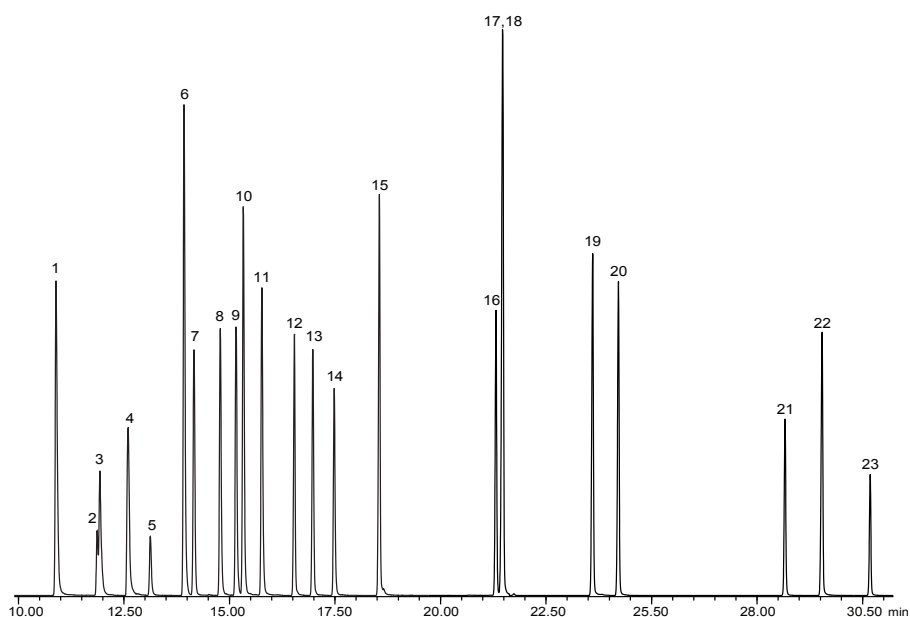

Figure 4.6 GC/MS SIM chromatogram for a standard mixture of quinolines and substituted anilines.(1) p-Chloroaniline; (2) Quinoline D-7(IS); (3) Quinoline; (4) Iso-Q; (5) 1-Indanone (VS); (6) 2-MQ; (7) 8-MQ; (8) 1-Iso-MQ; (9) 6-MQ; (10) 3-MQ; (11) 4-MQ; (12) o-NA; (13) 2,4-DMQ; (14) 2,6-DMQ; (15) m-NA; (16) 5-Cl-2-NA; (17) p-NA; (18) 2-Cl-5-NA; (19) 2-Cl-4-NA; (20) 2,6-DiCl-4-NA, (21) 6-Cl-2,4-DNA; (22) 2,4-DNA; (23) 2-Br-4,6-DNA.

4.3.3 Electron ionization

Different ion source temperatures were applied in order to choose the best analysis conditions. A high temperature of the ion source (over the default ion source operating temperature of 230 ˚C) can offer significant advantages such as improvement in peak shape, tailing reduction and signal increase, but it comes with the price of shorter filament lifetime [118]. An increase in electron ionization (EI) source temperature could also affect the screening process, since the spectra of some compounds may exhibit changes in high-mass fragment ion intensities, owing to the higher thermal energy, and/or there might be losses in signal for important higher mass confirming ions, changes in fragments ratios, or increase in interfering ions. Since most mass spectral libraries are acquired at low source temperatures or using other sample introduction techniques than GC (e.g. direct insertion probe), some loss in the libraries matching factor may occur when temperature is raised. The impact on the analysis of an increase in ion source temperature was investigated. The GC/MS analysis was run using a standard solution of the analytes of interest at different ion source temperatures: 280 °C (20 °C below the highest temperature of the GC oven), 300 °C, 325 °C, and 350 °C. The results were examined regarding the mass spectra to verify that they kept the same fragment ratios, and the chromatographic peaks with respect to absolute and relative abundances, and their shapes. A temperature of 300 and 325 °C showed a slight improvement in response and peak shape, as less tailing was present compared to 280 °C, while at 350 °C the response was lower due to increase of the noise. Hence 300 °C was the temperature of choice.

4.4   Method validation

Recoveries were determined for the whole sample preparation step by spiking a blank sample (a textile free of the compounds of interest)

with standard solution at two different concentration levels, and the procedure was run in triplicate. LODs and limit of quantifications (LOQs) were determined from five blank analyses as the signal of the blank plus 3 times and 10 times the standard deviation respectively.

Deuterated internal standard was spiked into the sample prior to the extraction procedure to correct for losses.

For HPLC/MS/MS analysis extraction solvent was used as procedural blank.

For the quantification of quinolines, two calibration curves, at low and high concentration level, with six points each, and without sample matrix, were used. To determine the matrix effects, extracts of blank samples were fortified with standard solution at two concentration levels. By comparing the response factors for pure standard and standard spiked with textile extracts the matrix effect was shown to be between 85% and 101% for all the compounds, which is to be considered small.

Precision and accuracy of the quantification method were estimated RSD and relative errors of spiked samples at two concentration levels. Blanks were run before and alongside each set of samples to check for contamination and memory effects.

4.5   Screening (Paper I)

Some methods on rapid screening and identification of multi-class substances of very high concern including phthalates, organotins, perfluoro chemicals, and flame retardants in textiles have recently been developed [119].

Non-targeted screening is an arduous task to carry out, especially when compounds are present at low concentration in the samples. Non-targeted screening means that non-selective extractions or clean-up methods are applied, and SIM or selected reaction monitoring (SRM)

acquisition mode cannot be used. Different solvents were tested for extraction and DCM gave rise to a larger number of peaks, allowing the extraction of non-polar as well as slightly polar compounds. In order to prove the applicability of the method on different classes of compounds, extraction and clean-up were tested on a group of twenty tentatively identified compounds with a wide polarity range (log K_{ow} 0.56 - 8.49). Blank samples were spiked with a known concentration of the analytes before extraction and used to calculate the recoveries. Linearity was checked spiking a blank extract with a standard mixture at five concentration levels, using Phenantrene as internal standard. Recoveries were between 100 and 110% (n=3) with a relative standard deviation <15%, while R^2 ranged from 0.994 to 0.999. LOD and LOQ were calculated using the formula: LOD = 3.3*(Sy/S) and LOQ = 10*(Sy/S), respectively, where Sy represents the standard deviation of the response and S the slope from the calibration curve.

For evaluation and quantification of the target analytes GC/MS was operating in SIM mode.

For screening purposes GC/MS in 70 eV EI mode and full scan (50-500 m/z range) acquisition was used. EI at standard setting gives a reproducible fragmentation of the molecules, and the obtained spectra are suitable for comparison with published libraries[120].

4.5.1 AMDIS

When little or no sample clean-up is used, matrix components can decrease the matching factor during comparison with libraries or give false identification by giving rise to unreleated peaks. The risk of both false positives and false negatives grows with the increase of the matrix complexity. Thus, it becomes fundamental to "clean" the spectra from interfering peaks, which can be done by manually subtracting the noise and background as well as checking the peak purity. The drawbacks of this operation are the amount of time needed, uneven background noise

in different parts of the chromatogram, and difficulties in separation of the different components in case of coeluting compounds.

The use of automatic processing by computer software reduces the time and effort. In this case the AMDIS (Automated Mass Spectral Deconvolution and Identification System) deconvolution software was used in combination with the NIST (National Institute of Standards and Technology Spectral Library) Mass Spectral Libraries.

The program extracts pure component spectra and matches them with compounds in selected reference libraries. For spectrum extraction the software uses the "model peak" method of Droomey [121], which by a least-squares procedure extract sets of ions, whose abundances are correlated to each other and have the same peak shape, suggesting to belong to the same component. In order to extract weak signals, the ion abundances are processed in signal-to-noise units rather than absolute units.

The total process involves the following steps:

1. Noise analysis and background subtraction
2. Component perception
3. Spectral deconvolution
4. Compound identification (using "pure" deconvoluted spectra).

The program then reports a match factor for the comparison against standard mass spectra in the NIST library. A threshold match factor filter, together with standard retention time (when available) can be used to fine tune the identifications.

4.5.2 Software parameters

A set of software was used to handle and evaluate raw data from the GC/MS analyses. The Agilent GC/MS workstation was used to register, store and handle raw data. AMDIS software, in combination with the NIST08 spectral library, as well as other dedicated libraries, was applied

for pretreatment and deconvolution of raw data. To allow a deeper search into the chromatogram (i.e. a lower concentration of present compounds can be identified), the parameter for components to be searched below each peak was set to 20, with medium resolution and high sensitivity. Confidence threshold for peaks identification against libraries was initially set to a relatively low match factor of 60%. Information of tentatively identified compounds, together with peak area, retention time, peak purity and net score were extracted from AMDIS as FIN files and implemented with boiling point and Kovats index data for each of the founds. MATLAB scripts were used to pool the collected data in tables. Since the World Anti Doping Agency set as identification criteria a variation in the retention time (RT) of an analyte to not differ by more than two 2% percent or ±0.1 minutes (whichever is smaller), a relative error up to 1% in the retention time, comparing to standard reference, was used. When reference standards were not available MATLAB scripts were used to list a set of candidate substances, searching for matches of identified compounds and retention time in the different samples, with a threshold of 0.1 min variation among the different samples and a match factor filter of 70%. The minimum number of samples in which a compound has to be identified in order to be included in the analysis was fixed to two.

The candidate list was then compared against the *Substance of very high concern* (SVHC) compiled by REACH. Health hazard and toxicity were checked in ChemSpider, Toxnet and other search engines available on the internet.

However, GC has some limitations for the analysis of SVHCs, e.g. for organotins, GC inevitably includes a derivatization step that affects the accuracy and precision, especially for the analysis of complex biological matrices [122], while for most brominated flame retardants, the high temperature of GC may lead to their degradation [123].

5. Results

5.1   Non targeted screening (Paper I)

Results from non-targeted screening showed more than hundred chemicals tentatively identified. The list of candidate compounds include substances of diverse functionalities, among them stabilizers, lubricants, plasticizers, solvents, biocides, and intermediates of resinous products and azo-dyes. Hydrocarbons (C_{11}-C_{28}), aliphatic alcohols (C_{11} and C_{20}), carboxylic acids (C_9 and C_{18}), esters of carboxylic acids, phthalate esters, aromatic and aliphatic amine, aromatic compounds appeared with higher frequency in the fifty-eight textile samples analyzed. Risks related to the detected substances were skin sensitization and irritation, reproduction toxicity, and proved or suspected carcinogenicity. Seven of the tentatively identified compounds, mainly phthalates toxic for reproduction, but also aromatic amine such as 4, 4'-methylenebis [2-methylaniline], o-toluidine, which are carcinogenic, were present in the SVHC list of the REACH regulation.

Since low resolution mass spectra alone might lead to false identifications, library suggestions were confirmed by comparison of retention time with reference compounds when available.

Noteworthy identified compounds were quinolines, benzothiazoles (quantified respectively in **Paper II** and **Paper III**), and aromatic amines, three of which were also quantified: p-aminoanisole, found in only one sample, with a concentration of 0.75 µg/g of textile, 2-Chloro-4-nitroaniline and 2,6-Dichloro-4-nitroaniline with an average concentration of 39 ng/g and 33 ng/g respectively (**Paper I**). Other aromatic amines of high concern were considered for a target analysis method presented in **Paper V**.

Carboxylic acids, used in finishing processes to give a synthetic textile an antistatic and dirt-repellent finish, were found at high average concentration across all samples. These compounds do, however, not pose any serious hazard [124]. Saturated fatty alcohols have been found to enhance skin permeation, with a degree depending on the chain length, with decanol showing the maximum permeation value [125]. Dodecanol and other fatty alcohol might, thus, enhance the absorption of more harmful compounds through the skin. Decanol and undecanol, lauryl alcohol, tridecanol and myristyl alcohol may also cause erythema [125]. Several other compounds, which were suspected to be present in the investigated samples, were not included in the candidate list due to a library match factor below 70%. However some of these compounds could be considered for future studies due to their harmful properties.

5.2 Quinolines (Paper II and IV)

Quinoline was detected at quantifiable levels in almost 80% of all the investigated samples. Its amount represented more than 50% of the total content of quinoline derivatives in each fabric. Iso-Q was the most abundant derivative (13% of the total amount of quinolines), while 1-M-iso-Q was the least abundant detected compound (<1%). The concentration profile of quinoline derivatives in the different samples was rather similar, indicating that these compounds are added as mixture of isomers.

In **Figure 5.1** results from **Paper II** and **IV** for quinolines concentrations in different type of fibers are presented.

 It is clear from the histogram, that there is a difference in the concentration of quinolines between samples made of 100% polyester (PE) and blended samples with an amount of polyester higher than 75% (PE mix), compared to blended polyamide (PA Mix) or blended cotton (CT Mix) and pure cotton (CT) samples.
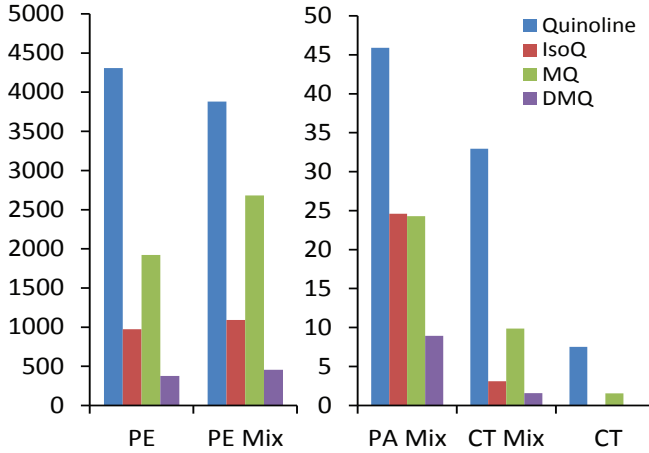
Figure 5.1 Average concentrations of quinolines (ng/g of textile) in different types of fiber

The average concentration of quinolines in polyester samples was 100 times higher than that in the blended garments made mostly of polyamide, and almost 800 times higher than that in garments made exclusively from cotton. The average quinoline concentration was around 2 µg/g, with the highest value (24 µg/g) found in a pink top made of 88% polyester. The Box and Whisker plot in **Figure 5.2** shows the distribution of quinolines (expressed as the sum of all derivatives in ng/g) in different types of fiber.
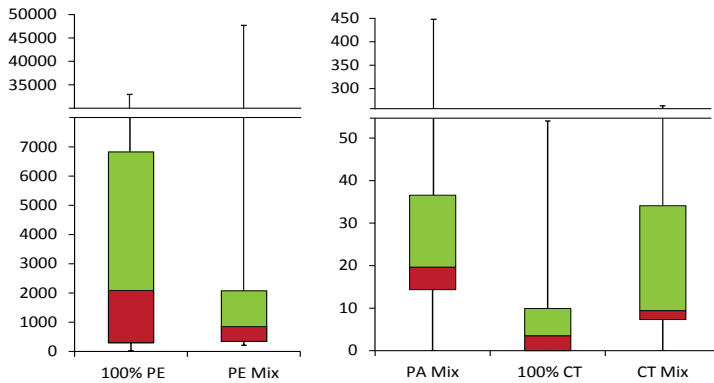

Figure 5.2 Boxplot of total quinolines concentration (ng/g) in different types of fiber with whiskers from minimum to maximum

31

5.3 Benzothiazoles and Benzotriazoles (Paper III and IV)

BT was the target compound with highest detection frequency >85%. Its average concentration was 0.5 µg/g, and the highest value, 50 µg/g, was found in a sample made of 100% polyester. The average concentration of MTBT was lower, 0.3µg/g, with a maximum of 1.7 µg/g in a blended polyester sample (**Paper III**). All the garments showed a similar profile with higher contents of BTs compared to BTri derivatives. Two BUVSs, UV-234 and UV-P, were detected in more than 50% of the samples. These compounds are both used as UV stabilizers in textiles, while UV-328, detected in only two samples might occur in textiles as a contaminant from, e.g., plastic products.

Average concentrations of BTs and BTris expressed in ng/g are presented in **Figure 5.3**.
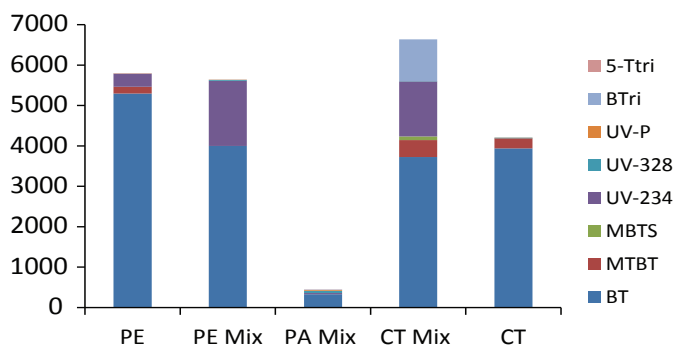

Figure 5.3 Average concentrations of Benzothiazoles and Benzotri-azoles (ng/g) in different types of fiber

It is interesting to note that three of the four garments made of "100 % organic cotton" and branded with "ecolabels" contained BT, as well as MTBT, with concentrations 7 to 30 times higher than the median concentration of the "ordinary" 100 % cotton garments. This suggests that "eco-labelling" is no guarantee that textiles are free from harmful chemicals.

5.4 Aromatic amines (Paper V)

Among the eleven substituted anilines, nine were detected in a set of seven samples, with the dinitroanilines showing a concentration one order of magnitude higher than that of the other target analytes.

2,6-DiCl-4-NA, which according to the EC regulation 1272/2008 is classified as being very toxic, was detected in four samples, with an average concentration of 3.70 µg/g and a maximum value of 19.9 µg/g in a black T-shirt 100% polyester. 6-Cl-2,4-DNA was detected in all the samples with an average concentration of 135 µg/g and a maximum concentration of 576 µg/g in a pair of black shorts made of 88% polyester. The same sample showed the highest number of detected compounds and the highest concentration for 2-Br-4,6-DNA (401 µg/g). A pair of black tracksuit bottoms for kids in polyester, showed as well high concentration of all the compounds and the maximum value for *p*-NA, 18µg/g.

5.5 Relationship with material

Results for the three groups of compounds, quinolines, benzothiazoles and benzotriazole, presented in **Paper II**, **III** and **IV** were subjected to multivariate statistical analysis to explore pattern differences (compounds which were not present in all the works, like MTBT and MBTS, were excluded). Principal component analysis (PCA) was performed to search for possible correlations with color, material, brand and place of origin. Results on the raw data, without any pretreatment, showed the target analytes being present to a greater extent in polyester materials compared to cotton based textile. When normalization, scaling and median centering were made on the data, PCA still indicated a correlation with the material used, **Figure 5.4**. Samples made of 100% polyester, or blended samples with polyester percentage >80%, were distinguished from the others. The loadings plot shows that, with an
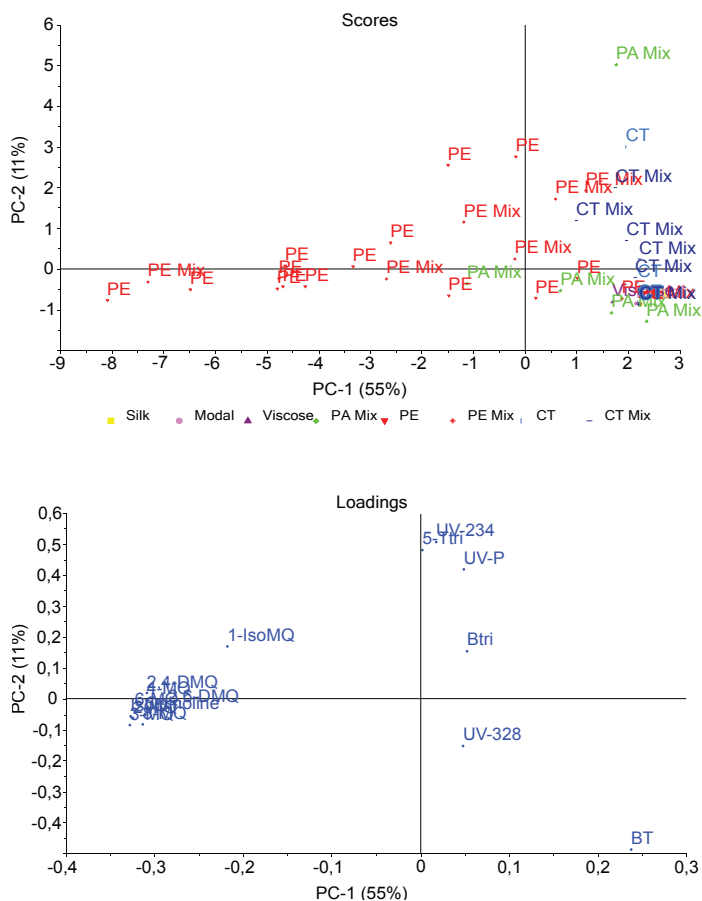
Figure 5.4 Principal component analysis (PCA) Scores (top) and Loading (bottom) plots of quinolines, benzothiazoles and benzotriazole in samples made of 100% polyester (PE), blended polyester >80% (PE Mix), blended polyamide >75% (PA Mix), blended cotton >70% (CT Mix), 100% cotton (CT).

explained variance of 55%, quinolines had the strongest influence on clustering polyester samples in the scores plot; furthermore, quinoline derivatives concentrations were strongly correlated between each other, while benzothiazole had more influence on clustering cotton garments, polyester garments were more spread out. This can suggest a dependence of target compounds with the material used. For quinolines, a possible explanation is that they are the base for making disperse dyes, which

are used to color synthetic textile materials based on polyester, acrylic, acetate and polyamide fibers [126].

Differences in the average concentration of quinolines and BT in polyester and cotton samples were found to be statistically significant using t-test for unequal variances, with p equal to 0.01 and 0.08 respectively (in case of BT, organic cotton samples were excluded).

5.6 Wash out of chemicals (Paper IV)

Quinoline, benzothiazole, benzotriazole and derivative compounds are sparingly soluble in water but more easily soluble in hot water. Laundry can, thus, be a route of emission into the environment of contaminants present in textile.

Analyses on samples before and after five and ten times washing were directed to quantify that emissions. Results showed an average loss of more than 50% for benzothiazole, whilst quinoline revealed a lower washout effect, probably due to the different usage, thus a diverse interaction with textile fibers. In **Figure 5.5** the average percentage of loss after five and ten washings is presented. Paired t-test showed that loss to be statistically significant for many of the investigated compounds ($p<0.05$).
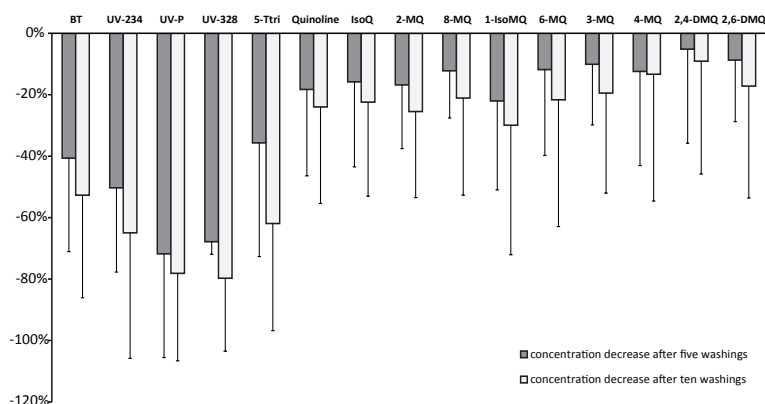

Figure 5.5 Percentage decrease in average concentration after five and ten washing steps for selected compounds.

To estimate the emission into household wastewater for 5 kg of clothes, the average concentration of quinoline and benzothiazole and their respective average loss were used. In this way the amount of released benzothiazole was calculated to 0.5 g and the amount of quinoline 0.24 g. This suggests that laundry is a source of emission of these compounds into household wastewater. The loss of some compounds, e.g. quinolines, was slow (20% after ten washings), demonstrating that significant amounts of the chemicals remain in the clothes for a long time and thus have the potential of a chronic impact on human health.

6. Conclusions and future perspectives

Focus has been put on the release of hazardous chemicals into the environment and there is today a well-known environmental problem caused by the textile industry. However, substances remaining in finished textile products, in daily close contact with the body, have been neglected as a potential risk to human health.

In this thesis work a method for explorative screening of textile materials, with special emphasis on clothes, have been developed. Over hundred compounds were tentatively identified and more than forty were confirmed to be present in a set of sixty garments available on the Swedish as well as the global market. The hazards posed by the identified substances were primarily skin sensitization and irritation, but also reproduction toxicity, and proved or suspected carcinogenicity. Seven of the tentatively identified compounds were present in the SVHC list of the REACH regulation.

Targeted analytical methods were developed for four groups of compounds: quinolines, benzothiazoles, benzotriazoles and aromatic amines, and were applied on the textile materials.

Concentration differences for some of the target analytes was found to be related to the fiber type used in the textile. In particular, higher amounts of quinolines and benzothiazole were detected in textile manufactured mainly from polyester. Moreover, it is indicated that organic cotton and eco labelling are no guarantee that textiles are free from harmful chemicals.

The release of these two groups of compounds during washing of the clothing textiles has been demonstrated. A discharge into household wastewater and further on to the aquatic environment is a most likely consequence.

For compounds with a slow release during washing, a significant amount remain in the clothes have the possibility for a long term human exposure.

Chemical residues from textiles have the potential to migrate from clothes to the human skin and be absorbed according to their size and octanol/water partition coefficient, and may thus cause local and/or systemic effects. Harmless compounds or compounds with minor health effect could be metabolized by bacteria present on the skin, or if absorbed, be converted to harmful substances by hepatic enzyme systems. A combination of different toxic compounds could also enhance (or reduce) the health risk of the single substances.

Future research:

In order to get a better picture of the total exposure, not only clothes, but several other textile articles present in the indoor environment should be analyzed.

Screening and targeting methods using LC/MS/MS with high resolution, using positive and negative electrospray ionization, as well as APCI, should be developed to include more hydrophilic compounds, which are more likely to be washed out and end up not in the sludge but rather go right into the aquatic system.

Candidate compounds for further research has to be selected by combining explorative chemical analysis with data on skin penetrating properties and toxicological effects, to put a special focus on compounds with a potential for human exposure.

Transfer of the selected compounds from textile to the skin as well as their absorption needs to be investigated. Extraction could be made using simulated sweat and bioassay toxicity test made on the chemicals released from the fabric.

Further research should also be directed towards analytical methods for determination of identified textile chemicals and/or their metabolites in human blood and/or urine.

Methods for treating the textiles (e.g. carbon dioxide cleaners) to avoid having these compounds spreading into the environment should also be investigated.

7. Acknowledgments

In these fantastic five years many people crossed their lives with mine for a brief moment and many others walked along my side till the end, to all of them I would like to send my deepest gratitude, for what they gave and took from me, and for making this journey enjoyable in a place that I started to call home.

First, I would like to thank the Department of Environmental Science and Analytical Chemistry, at Stockholm University, for accepting me as PhD student and for financial support.

I would like to individually acknowledge the people who made this thesis work possible:

My main supervisor, **Conny** Östman for giving me the opportunity to work on this project, leading me through it, encouraging and pushing me to do always my best, for being there when I needed it most, and keeping trying to teach me the way of thinking positive.

My second supervisor, **Gunnar** Thorsén for the boundless, enthusiastic, tremendous love for science, with which he likes to infect people; for the help in the project and in the AK kurs, and for caring so much.

My third supervisor, **Carlo** Crescenzi for being the very first one to believe in me, for his kind and precious help with experiments, for sharing his broad knowledge and the strong and genuine passion for chemistry, which I really admire.

Ulrika Nilsson for the nice discussions and the kind help. Your intellectual curiosity is a model for the students. You are a teacher of chemistry and life!

Rozanna Avagyan. Thanks for the nice collaboration in the textile project, the huge help, support and friendship.

Francesco Iadaresta for collaboration, good lab work and great ideas, which really pushed forward the project.

Pau Benetó Vallés for the invaluable help with MATLAB script in Paper I and comments on the thesis.

Moreover I would like to thank all the people who proof read my thesis and gave me comments, **Anders** Colmsjö, **Roger** Westerholm, **Leopold** Ilag, for coments on the thesis and all the nice scientific/philosophical/

cultural discussions, and making me discover my true color, **Magnus** Åberg, for the explanation about Tukey test; your help was statistically significant! **Christoffer** Bergvall for being a generous friend, always eager to help. **Ingrid** Granelli, for patiently organizing the seminars.

I would like to show my gratitude to **AnneMarie** Nilsson Hagelroth and **Lena** Elfver, for always being so nice, **Jonas** Rutberg, för att hälpa mig med "In design" och många andra saker, **Karin** Lidén, tack för att hjälpa mig att öva svenska, det är kul att prata med dig, **Michael** Strandellan for help with instruments and friendship, **Jan** Holmbäck, **Bengt** Herslöf, and **Mohamed** Abdel-Rehim.

I would like to thank the colleagues I shared the office with: **Thuy** TTran, a super sweet friend, **Petter** Olsson, for all the distressing laughs and friendship, **Erik** Tengstrand, for cheering me up with cats pictures, **Nadia** Kiselova, for filling my desk with encouraging messages and for having a wise advise in every situation, **Pedro** Sousa for help with statistical analysis.

All the PhD students and staff at the Department:

Ioannis Sadiktsis, for fixing all the problems I had in the lab and for invaluable comments on the thesis and manuscript, **Alessandro** Quaranta for the help with language corrections and for amusing me with all the colorful, epic, vivid, picturesque as well as horrifying jokes, **Franciccio** Iadaresta (again), thank you for always being by my side in the darkest days, I couldn't have gone through everything without your friendship, **Javier** Zurita, for all the fun moments and being the best Spanish teacher (no se lo digas a Pau), **Hwanmi** Lim for her sweetness, **Farshid** Mashayekhy Rad, for the nice company during the weekends of work, **Ahmed** Ramzy, for the contagious smile and the generosity, **Trifa** Mohammad Ahmed, for taking precious time for reading my thesis and always kindly helping me (good luck with your dissertation, you'll do it great!), **Rozanna** Superwoman Avagyan (again), you have a truly gentle heart and **Jonas** Fyrestam for the nice conversations (the other people can say we are boring just because they don't know all the crazy things we do after job!), **Rudolf** fisherman Andrýs, **Anna** Sroka-Bartnicka for the nice conversations, **Aziza** El Beqqali, **Hatem** Elmongy, **Jessica** Norrgran Engdahl for tips on the thesis, **Ioannis** Athanassiadis for providing NIST library.

The former PhD students: First of all **Caroline** Bergh for teaching me all I know about GC and being so helpful during a period, which

8. References

1. Sarayu, K. and S. Sandhya, *Current Technologies for Biological Treatment of Textile Wastewater–A Review.* Applied Biochemistry and Biotechnology, 2012. **167**(3): p. 645-661.

2. Correia, V.M., T. Stephenson, and S.J. Judd, *Characterisation of textile wastewaters - a review.* Environmental Technology, 1994. **15**(10): p. 917-929.

3. Lexén, K., C. de Wit, B. Jansson, L.-O. Kjeller, S.-E. Kulp, K. Ljung, G. Söderström, and C. Rappe, *Polychlorinated dibenzo-p-dioxin and dibenzofuran levels and patterns in samples from different Swedish industries analyzed within the Swedish dioxin survey.* Chemosphere, 1993. **27**(1–3): p. 163-170.

4. Parliament, E. and t.C.o.t.E. Union, *Regulation (EC) no. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the registration, evaluation, authorization and restriction of chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) no. 793/93 and Commission Regulation (EC) no. 1488/94, as well as Council Directive 76/769/EEC and commission Directives 91/155/EEC, 93/67/EEC, 93/105/CE and 2000/21/EC.* Official Journal of the European Union, 2006. **396**.

5. Deguine, J.-P., P. Ferron, and D. Russell, *Sustainable Pest Management for Cotton Production: A Review*, in *Sustainable Agriculture*, E. Lichtfouse, M. Navarrete, P. Debaeke, S. Véronique, and C. Alberola, Editors. 2009, Springer Netherlands. p. 411-442.

6. Beynon, S.A., *Potential environmental consequences of administration of ectoparasiticides to sheep.* Veterinary Parasitology, 2012. **189**(1): p. 125-135.

7. Gebefügi, I., *Chemical exposure in enclosed environments.* Toxicological & Environmental Chemistry, 1989. **20-21**(1): p. 121-127.

8. Lacasse, K. and W. Baumann, *Textile Chemicals: Environmental Data and Facts*2004: Springer.

9. Sando, Y., E. Nakano, H. Ishidoshiro, and K. Sando, *Method and apparatus for the pretreatment of a cloth*, 1995, Google Patents.

10. Chen, H.-L. and L.D. Burns, *Environmental analysis of textile products.* Clothing and Textiles Research Journal, 2006. **24**(3): p. 248-261.

11. Adrian, K. and G. Rosch, *Oxidative desizing agent and process for oxidative desizing*, 1982, Google Patents.

12. Liu, J., S. Salmon, and G. Wu, *Desizing and Scouring Process*, 2009, Google Patents.

13. James, A.C. and G.S. Harry, *Method of bleaching*, 1936, Google Patents.

14. Zeronian, S. and M. Inglesby, *Bleaching of cellulose by hydrogen peroxide.* Cellulose, 1995. **2**(4): p. 265-272.

15. Frederick, B.V., *Method of treating textiles*, 1927, Google Patents.

16. Huang, C. and R.K. Schlatzer, *Water thickening agents consisting of copolymers of crosslinked acrylic acids and esters*, 1985, Google Patents.

17. Chung, K.-T. and C.E. Cerniglia, *Mutagenicity of azo dyes: Structure-activity relationships.* Mutation Research/Reviews in Genetic Toxicology, 1992. **277**(3): p. 201-220.

18. Hunger, K., *Industrial dyes.* Chemistry, properties, application, 2003.

19. Yahagi, T., M. Degawa, Y. Seino, T. Matsushima, M. Nagao, T. Sugimura, and Y. Hashimoto, *Mutagenicity of carcinogenic azo dyes and their derivatives.* Cancer Letters, 1975. **1**: p. 91-96.

20. Pinheiro, H.M., E. Touraud, and O. Thomas, *Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters.* Dyes and Pigments, 2004. **61**(2): p. 121-139.

21. Chung, K.-T., *The significance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes.* Mutation Research/Reviews in Genetic Toxicology, 1983. **114**(3): p. 269-281.

22. Puvaneswari, N., J. Muthukrishnan, and P. Gunasekaran, *Toxicity assessment and microbial degradation of azo dyes.* Indian journal of experimental biology, 2006. **44**(8): p. 618.

23. Luo, H., X.-a. Ning, X. Liang, Y. Feng, and J. Liu, *Effects of sawdust-CPAM on textile dyeing sludge dewaterability and filter cake properties.* Bioresource Technology, 2013. **139**: p. 330-336.

24. Bajaj, P., *Finishing of textile materials.* Journal of Applied Polymer Science, 2002. **83**(3): p. 631-659.

25. Le Marechal, A.M., B. Križanec, S. Vajnhandl, and J.V. Valh. *Textile finishing industry as an important source of organic pollutants*. in *Organic Pollutants Ten Years After the Stockholm Convention-Environmental and Analytical Update*. 2012.

26. Vanhulle, S., M. Trovaslet, E. Enaud, M. Lucas, S. Taghavi, D. van der Lelie, B. van Aken, M. Foret, R.C.A. Onderwater, D. Wesenberg, S.N. Agathos, Y.-J. Schneider, and A.-M. Corbisier, *Decolorization, Cytotoxicity, and Genotoxicity Reduction During a Combined*

Ozonation/Fungal Treatment of Dye-Contaminated Wastewater. Environmental Science & Technology, 2008. **42**(2): p. 584-589.

27. Ning, X.-A., M.-Q. Lin, L.-Z. Shen, J.-H. Zhang, J.-Y. Wang, Y.-J. Wang, Z.-Y. Yang, and J.-Y. Liu, *Levels, composition profiles and risk assessment of polycyclic aromatic hydrocarbons (PAHs) in sludge from ten textile dyeing plants.* Environmental research, 2014. **132**: p. 112-118.

28. Liang, X., X.-a. Ning, G. Chen, M. Lin, J. Liu, and Y. Wang, *Concentrations and speciation of heavy metals in sludge from nine textile dyeing plants.* Ecotoxicology and Environmental Safety, 2013. **98**: p. 128-134.

29. Preiss, A., U. Sänger, N. Karfich, K. Levsen, and C. Mügge, *Characterization of Dyes and Other Pollutants in the Effluent of a Textile Company by LC/NMR and LC/MS.* Analytical Chemistry, 2000. **72**(5): p. 992-998.

30. Loos, R., G. Hanke, G. Umlauf, and S.J. Eisenreich, *LC–MS–MS analysis and occurrence of octyl- and nonylphenol, their ethoxylates and their carboxylates in Belgian and Italian textile industry, waste water treatment plant effluents and surface waters.* Chemosphere, 2007. **66**(4): p. 690-699.

31. Berryman, D., F. Houde, C. DeBlois, and M. O'Shea, *Nonylphenolic compounds in drinking and surface waters downstream of treated textile and pulp and paper effluents: a survey and preliminary assessment of their potential effects on public health and aquatic life.* Chemosphere, 2004. **56**(3): p. 247-255.

32. Abad, E., M.A. Adrados, J. Caixach, B. Fabrellas, and J. Rivera, *Dioxin mass balance in a municipal waste incinerator.* Chemosphere, 2000. **40**(9–11): p. 1143-1147.

33. Dave, G. and P. Aspegren, *Comparative toxicity of leachates from 52 textiles to Daphnia magna.* Ecotoxicology and Environmental Safety, 2010. **73**(7): p. 1629-1632.

34. Gómez, N., M.V. Sierra, A. Cortelezzi, and A. Rodrigues Capítulo, *Effects of discharges from the textile industry on the biotic integrity of benthic assemblages.* Ecotoxicology and Environmental Safety, 2008. **69**(3): p. 472-479.

35. Windler, L., M. Height, and B. Nowack, *Comparative evaluation of antimicrobials for textile applications.* Environment International, 2013. **53**: p. 62-73.

36. Kajiwara, N., M. Sueoka, T. Ohiwa, and H. Takigami, *Determination of flame-retardant hexabromocyclododecane diastereomers in textiles.* Chemosphere, 2009. **74**(11): p. 1485-1489.

37.   Zhu, F., W. Ruan, M. He, F. Zeng, T. Luan, Y. Tong, T. Lu, and G. Ouyang, *Application of solid-phase microextraction for the determination of organophosphorus pesticides in textiles by gas chromatography with mass spectrometry.* Analytica chimica acta, 2009. **650**(2): p. 202-206.

38.   Vestergren, R., D. Herzke, T. Wang, and I.T. Cousins, *Are imported consumer products an important diffuse source of PFASs to the Norwegian environment?* Environmental Pollution, 2015. **198**: p. 223-230.

39.   Buck, R.C., J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, P. de Voogt, A.A. Jensen, K. Kannan, S.A. Mabury, and S.P. van Leeuwen, *Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins.* Integrated environmental assessment and management, 2011. **7**(4): p. 513-541.

40.   Herzke, D., E. Olsson, and S. Posner, *Perfluoroalkyl and polyfluoroalkyl substances (PFASs) in consumer products in Norway – A pilot study.* Chemosphere, 2012. **88**(8): p. 980-987.

41.   Wang, Y., Z. Zeng, and M. Liu, *Analysis of naphthalene residues in textile samples by GC-FID using sol-gel-derived SPME fiber.* Journal of chromatographic science, 2011. **49**(1): p. 29-34.

42.   Padban, N. and I. Odenbrand, *Polynuclear Aromatic Hydrocarbons in Fly Ash from Pressurized Fluidized Bed Gasification of Fuel Blends. A Discussion of the Contribution of Textile to PAHs.* Energy & Fuels, 1999. **13**(5): p. 1067-1073.

43.   Chen, L., H. Jin, L. Wang, L. Sun, H. Xu, L. Ding, A. Yu, and H. Zhang, *Dynamic ultrasound-assisted extraction coupled on-line with solid support derivatization and high-performance liquid chromatography for the determination of formaldehyde in textiles.* Journal of Chromatography A, 2008. **1192**(1): p. 89-94.

44.   Brigden, K., D. Santillo, and P. Johnston, *Nonylphenol ethoxylates (NPEs) in textile products, and their release through laundering.* Greenpeace Research Laboratories Technical Report, 2012. **1**.

45.   Cioni, F., G. Bartolucci, G. Pieraccini, S. Meloni, and G. Moneti, *Development of a solid phase microextraction method for detection of the use of banned azo dyes in coloured textiles and leather.* Rapid Communications in Mass Spectrometry, 1999. **13**(18): p. 1833-1837.

46.   Ahlström, L.-H., C. Sparr Eskilsson, and E. Björklund, *Determination of banned azo dyes in consumer goods.* TrAC Trends in Analytical Chemistry, 2005. **24**(1): p. 49-56.

47.   Horstmann, M. and M.S. McLachlan, *Textiles as a source of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) in human skin and sewage sludge.* Environmental Science and Pollution Research, 1994. **1**(1): p. 15-20.

48. Klasmeier, J. and M.S. McLachlan, *PCDD/Fs in textiles — Part 1: A screening method for detection of octachlorodibenzo-p-dioxin and octachlorodibenzofuran.* Chemosphere, 1998. **36**(7): p. 1627-1635.

49. Križanec, B., A.M. Le Marechal, E. Vončina, and D. Brodnjak-Vončina, *Presence of dioxins in textile dyes and their fate during the dyeing processes.* Acta Chimica Slovenica, 2005. **52**: p. 111-118.

50. Sedlak, D., R. Dumler-Gradl, H. Thoma, and O. Vierle, *Polyhalogenated dibenzo-p-dioxins and dibenzofurans in the exhaust air during textile processings.* Chemosphere, 1998. **37**(9–12): p. 2071-2076.

51. Moltó, J., R. Font, and J.A. Conesa, *Study of the Organic Compounds Produced in the Pyrolysis and Combustion of Used Polyester Fabrics.* Energy & Fuels, 2006. **20**(5): p. 1951-1958.

52. SARTORELLI, P., C. APREA, A. CENNI, M.T. NOVELLI, D. ORSI, S. PALMI, and G. MATTEUCCI, *Prediction of Percutaneous Absorption from Physicochemical Data: A Model Based on Data of In Vitro Experiments.* Annals of Occupational Hygiene, 1998. **42**(4): p. 267-276.

53. Bos, J.D. and M.M. Meinardi, *The 500 Dalton rule for the skin penetration of chemical compounds and drugs.* Experimental dermatology, 2000. **9**(3): p. 165-169.

54. Kezic, S. and J.B. Nielsen, *Absorption of chemicals through compromised skin.* International Archives of Occupational and Environmental Health, 2009. **82**(6): p. 677-688.

55. Klasmeier, J., A. Mühlebach, and M.S. McLachlan, *PCDD/Fs in textiles — Part II: Transfer from clothing to human skin.* Chemosphere, 1999. **38**(1): p. 97-108.

56. Gallagher, M., C.J. Wysocki, J.J. Leyden, A.I. Spielman, X. Sun, and G. Preti, *Analyses of volatile organic compounds from human skin.* British Journal of Dermatology, 2008. **159**(4): p. 780-791.

57. Blum, A., M. Gold, B. Ames, F. Jones, E. Hett, R. Dougherty, E. Horning, I. Dzidic, D. Carroll, R. Stillwell, and J. Thenot, *Children absorb tris-BP flame retardant from sleepwear: urine contains the mutagenic metabolite, 2,3-dibromopropanol.* Science, 1978. **201**(4360): p. 1020-1023.

58. Stahlmann, R., M. Wegner, K. Riecke, M. Kruse, and T. Platzek, *Sensitising potential of four textile dyes and some of their metabolites in a modified local lymph node assay.* Toxicology, 2006. **219**(1–3): p. 113-123.

59. Lensen, G., F. Jungbauer, M. Gonçalo, and P.J. Coenraads, *Airborne irritant contact dermatitis and conjunctivitis after occupational exposure to chlorothalonil in textiles.* Contact Dermatitis, 2007. **57**(3): p. 181-186.

60.   Steenland, K., T. Fletcher, and D.A. Savitz, *Epidemiologic Evidence on the Health Effects of Perfluorooctanoic Acid (PFOA).* Environmental Health Perspectives, 2010. **118**(8): p. 1100-1108.

61.   Galloway, T. and R. Handy, *Immunotoxicity of Organophosphorous Pesticides.* Ecotoxicology, 2003. **12**(1-4): p. 345-363.

62.   Boffetta, P., N. Jourenkova, and P. Gustavsson, *Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons.* Cancer Causes & Control, 1997. **8**(3): p. 444-472.

63.   McGregor, D.B., C. Partensky, J. Wilbourn, and J.M. Rice, *An IARC evaluation of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans as risk factors in human carcinogenesis.* Environmental Health Perspectives, 1998. **106**(Suppl 2): p. 755-760.

64.   Nimrod, A.C. and W.H. Benson, *Environmental estrogenic effects of alkylphenol ethoxylates.* CRC Critical Reviews in Toxicology, 1996. **26**(3): p. 335-364.

65.   Heudorf, U., V. Mersch-Sundermann, and J. Angerer, *Phthalates: Toxicology and exposure.* International Journal of Hygiene and Environmental Health, 2007. **210**(5): p. 623-634.

66.   Vezmar, M. and E. Georges, *Reversal of MRP-mediated doxorubicin resistance with quinoline-based drugs.* Biochemical Pharmacology, 2000. **59**(10): p. 1245-1252.

67.   Mukherjee, S. and M. Pal, *Quinolines: a new hope against inflammation.* Drug Discovery Today, 2013. **18**(7–8): p. 389-398.

68.   Lam, P.-l., C.-w. Kan, M.C.-w. Yuen, S.-y. Cheung, R. Gambari, K.-h. Lam, J.C.-o. Tang, and C.-h. Chui, *Studies on quinoline type dyes and their characterisation studies on acrylic fabric.* Coloration Technology, 2012. **128**(3): p. 192-198.

69.   Pereira, W.E., C.E. Rostad, J.R. Garbarino, and M.F. Hult, *Groundwater contamination by organic bases derived from coal-tar wastes.* Environmental Toxicology and Chemistry, 1983. **2**(3): p. 283-294.

70.   Atkinson, R., E.C. Tuazon, J. Arey, and S.M. Aschmann, *Atmospheric and indoor chemistry of gas-phase indole, quinoline, and isoquinoline.* Atmospheric Environment, 1995. **29**(23): p. 3423-3432.

71.   Dong, M., I. Schmeltz, E. Jacobs, and D. Hoffmann, *Aza-arenes in tobacco smoke.* Journal of Analytical Toxicology, 1978. **2**(1): p. 21-25.

72.   Leníček, J., M. Sekyra, K. Bednárková, I. Beneš, and F. Šípek, *Fractionation and chemical analysis of urban air particulate extracts.* International Journal of Environmental Analytical Chemistry, 2000. **77**(4): p. 269-288.

73.   Pearlman, R.S., S.H. Yalkowsky, and S. Banerjee, *Water Solubilities of Polynuclear Aromatic and Heteroaromatic Compounds*. Journal of Physical and Chemical Reference Data, 1984. **13**(2): p. 555-562.

74.   Wakeham, S.G., *Azaarenes in Recent lake sediments*. Environmental Science & Technology, 1979. **13**(9): p. 1118-1123.

75.   Stedman, R.L., *Chemical composition of tobacco and tobacco smoke*. Chemical Reviews, 1968. **68**(2): p. 153-207.

76.   Nielsen, T., P. Clausen, and F.P. Jensen, *Determination of basic azaarenes and polynuclear aromatic hydrocarbons in airborne particulate matter by gas chromatography*. Analytica Chimica Acta, 1986. **187**(0): p. 223-231.

77.   Drushel, H.V. and A.L. Sommers, *Isolation and Identification of Nitrogen Compounds in Petroleum*. Analytical Chemistry, 1966. **38**(1): p. 19-28.

78.   Brulik, J., Z. Simek, and P. de Voogt, *A new liquid chromatography–tandem mass spectrometry method using atmospheric pressure photo ionization for the simultaneous determination of azaarenes and azaarones in Dutch river sediments*. Journal of Chromatography A, 2013. **1294**(0): p. 33-40.

79.   de Voogt, P. and R.W.P.M. Laane, *Assessment of azaarenes and azaarones (oxidized azaarene derivatives) in the Dutch coastal zone of the North Sea*. Chemosphere, 2009. **76**(8): p. 1067-1074.

80.   Yuan, Y.-l., Y.-z. Wen, X.-y. Li, and S.-z. Luo, *Treatment of wastewater from dye manufacturing industry by coagulation*. Journal of Zhejiang University SCIENCE A, 2006. **7**(2): p. 340-344.

81.   Oliveira, D.P., P.A. Carneiro, M.K. Sakagami, M.V.B. Zanoni, and G.A. Umbuzeiro, *Chemical characterization of a dye processing plant effluent—Identification of the mutagenic components*. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 2007. **626**(1–2): p. 135-142.

82.   Asakura, S., S. Sawada, T. Sugihara, H. Daimon, and F. Sagami, *Quinoline-induced chromosome aberrations and sister chromatid exchanges in rat liver*. Environmental and Molecular Mutagenesis, 1997. **30**(4): p. 459-467.

83.   La Voie, E.J., S. Dolan, P. Little, C.X. Wang, S. Sugie, and A. Rivenson, *Carcinogenicity of quinoline, 4- and 8-methylquinoline and benzoquinolines in newborn mice and rats*. Food and Chemical Toxicology, 1988. **26**(7): p. 625-629.

84.   Hirao, K., Y. Shinohara, H. Tsuda, S. Fukushima, M. Takahashi, and N. Ito, *Carcinogenic Activity of Quinoline on Rat Liver*. Cancer Research, 1976. **36**(2 Part 1): p. 329-335.

85.   USA, E. *Quinoline. Integrated Risk Information System.* 2001; Available from: .http:www.epa.gov/iris/subst/1004.htm

86.   Ni, H.-G., F.-H. Lu, X.-L. Luo, H.-Y. Tian, and E.Y. Zeng, *Occurrence, Phase Distribution, and Mass Loadings of Benzothiazoles in Riverine Runoff of the Pearl River Delta, China.* Environmental Science & Technology, 2008. **42**(6): p. 1892-1897.

87.   Kloepfer, A., M. Jekel, and T. Reemtsma, *Determination of benzothiazoles from complex aqueous samples by liquid chromatography–mass spectrometry following solid-phase extraction.* Journal of Chromatography A, 2004. **1058**(1–2): p. 81-88.

88.   Weiss, S. and T. Reemtsma, *Determination of Benzotriazole Corrosion Inhibitors from Aqueous Environmental Samples by Liquid Chromatography-Electrospray Ionization-Tandem Mass Spectrometry.* Analytical Chemistry, 2005. **77**(22): p. 7415-7420.

89.   Kim, J.-W., B.R. Ramaswamy, K.-H. Chang, T. Isobe, and S. Tanabe, *Multiresidue analytical method for the determination of antimicrobials, preservatives, benzotriazole UV stabilizers, flame retardants and plasticizers in fish using ultra high performance liquid chromatography coupled with tandem mass spectrometry.* Journal of Chromatography A, 2011. **1218**(22): p. 3511-3520.

90.   Nakata, H., S. Murata, and J. Filatreau, *Occurrence and Concentrations of Benzotriazole UV Stabilizers in Marine Organisms and Sediments from the Ariake Sea, Japan.* Environmental Science & Technology, 2009. **43**(18): p. 6920-6926.

91.   Giger, W., C. Schaffner, and H.-P.E. Kohler, *Benzotriazole and Tolyltriazole as Aquatic Contaminants. 1. Input and Occurrence in Rivers and Lakes†.* Environmental Science & Technology, 2006. **40**(23): p. 7186-7192.

92.   Brorström-Lundén, E., K. Hansson, M. Remberger, L. Kaj, J. Magnér, and H. Andersson, *Screening of benzothiazoles, benzenediamines, dicyclohexylamine and benzotriazoles.* IVL Report B, 2011. **2023**: p. 1-53.

93.   Kloepfer, A., M. Jekel, and T. Reemtsma, *Occurrence, Sources, and Fate of Benzothiazoles in Municipal Wastewater Treatment Plants.* Environmental Science & Technology, 2005. **39**(10): p. 3792-3798.

94.   Avagyan, R., I. Sadiktsis, C. Bergvall, and R. Westerholm, *Tire tread wear particles in ambient air—a previously unknown source of human exposure to the biocide 2-mercaptobenzothiazole.* Environmental Science and Pollution Research, 2014. **21**(19): p. 11580-11586.

95.   Reemtsma, T., O. Fiehn, G. Kalnowski, and M. Jekel, *Microbial Transformations and Biological Effects of Fungicide-Derived Benzothiazoles Determined in Industrial Wastewater.* Environmental Science & Technology, 1995. **29**(2): p. 478-485.

96.   Jung, J.H., J.L. McLaughlin, J. Stannard, and J.D. Guin, *Isolation, via activity-directed fractionation, of mercaptobenzothiazole and dibenzothiazyl disulfide as 2 allergens responsible for tennis shoe dermatitis.* Contact Dermatitis, 1988. **19**(4): p. 254-259.

97.   Fishbein, L., *Municipal and Industrial Hazardous Waste Management: an Overview.* Toxicology and Industrial Health, 1991. **7**(5-6): p. 209-220.

98.   Tangtian, H., L. Bo, L. Wenhua, P.K.S. Shin, and R.S.S. Wu, *Estrogenic potential of benzotriazole on marine medaka (Oryzias melastigma).* Ecotoxicology and Environmental Safety, 2012. **80**(0): p. 327-332.

99.   Asimakopoulos, A.G., L. Wang, N.S. Thomaidis, and K. Kannan, *Benzotriazoles and benzothiazoles in human urine from several countries: A perspective on occurrence, biotransformation, and human exposure.* Environment International, 2013. **59**(0): p. 274-281.

100.   Kahl, T., K.-W. Schröder, F.R. Lawrence, W.J. Marshall, H. Höke, and R. Jäckh, *Aniline*, in *Ullmann's Encyclopedia of Industrial Chemistry*2000, Wiley-VCH Verlag GmbH & Co. KGaA.

101.   Landler, J. and E. Worfel, *Process for the preparation of water-soluble azo dyestuffs by reacting an aromatic amine and a coupling component with alkali metal nitrite or alkyl nitrite in the absence of acid*, 1977, Google Patents.

102.   Chung, K.-T., S.E. Stevens, and C.E. Cerniglia, *The reduction of azo dyes by the intestinal microflora.* Critical reviews in microbiology, 1992. **18**(3): p. 175-190.

103.   Platzek, T., C. Lang, G. Grohmann, U.-S. Gi, and W. Baltes, *Formation of a carcinogenic aromatic amine from an azo dye by human skin bacteria in vitro.* Human & Experimental Toxicology, 1999. **18**(9): p. 552-559.

104.   MANCUSO, T.F. and A.A. EL-ATTAR, *Cohort Study of Workers Exposed to Betanaphthylamine and Benzidine.* Journal of Occupational and Environmental Medicine, 1967. **9**(6): p. 277-285.

105.   Talaska, G., *Aromatic amines and human urinary bladder cancer: exposure sources and epidemiology.* Journal of Environmental Science and Health, Part C, 2003. **21**(1): p. 29-43.

106.   Rubino, G.F., G. Scansetti, G. Piolatto, and E. Pira, *The carcinogenic effect of aromatic amines: an epidemiological study on the role of o-toluidine and 4, 4'-methylene bis (2-methylaniline) in inducing bladder cancer in man.* Environmental research, 1982. **27**(2): p. 241-254.

107.   Júnior, D.S., F.J. Krug, M.d.G. Pereira, and M. Korn, *Currents on Ultrasound-Assisted Extraction for Sample Preparation and*

Spectroscopic Analytes Determination. Applied Spectroscopy Reviews, 2006. **41**(3): p. 305-321.

108. Avagyan, R., I. Sadiktsis, G. Thorsén, C. Östman, and R. Westerholm, *Determination of benzothiazole and benzotriazole derivates in tire and clothing textile samples by high performance liquid chromatography–electrospray ionization tandem mass spectrometry.* Journal of Chromatography A, 2013. **1307**(0): p. 119-125.

109. Hennion, M.-C., *Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography.* Journal of Chromatography A, 1999. **856**(1–2): p. 3-54.

110. Poole, C.F., *New trends in solid-phase extraction.* TrAC Trends in Analytical Chemistry, 2003. **22**(6): p. 362-373.

111. Forgács, E., *Retention characteristics and practical applications of carbon sorbents.* Journal of Chromatography A, 2002. **975**(2): p. 229-243.

112. West, C., C. Elfakir, and M. Lafosse, *Porous graphitic carbon: A versatile stationary phase for liquid chromatography.* Journal of Chromatography A, 2010. **1217**(19): p. 3201-3216.

113. Bruner, F., G. Crescentini, and F. Mangani, *Graphitized carbon black: A unique adsorbent for gas chromatography and related techniques.* Chromatographia, 1990. **30**(9-10): p. 565-572.

114. Hennion, M.-C., *Graphitized carbons for solid-phase extraction.* Journal of Chromatography A, 2000. **885**(1): p. 73-95.

115. Di Corcia, A., M. Marchetti, R. Samperi, and A. Marcomini, *Liquid chromatographic determination of linear alkylbenzenesulfonates in aqueous environmental samples.* Analytical Chemistry, 1991. **63**(11): p. 1179-1182.

116. Michel, M. and B. Buszewski, *Porous graphitic carbon sorbents in biomedical and environmental applications.* Adsorption, 2009. **15**(2): p. 193-202.

117. Di Corcia, A., R. Samperi, A. Marcomini, and S. Stelluto, *Graphitized carbon black extraction cartridges for monitoring polar pesticides in water.* Analytical Chemistry, 1993. **65**(7): p. 907-912.

118. Thomson, C., C. Broadbent, and H. Prest, *The 5975C Series MSDs: Guidanc e in implementing high ion source temperatures.* Agilent Technical Note, 2007.

119. Zhang, L., X. Luo, Z. Niu, X. Ye, Z. Tang, and P. Yao, *Rapid screening and identification of multi-class substances of very high concern in textiles using liquid chromatography-hybrid linear ion trap orbitrap mass spectrometry.* Journal of Chromatography A, 2015. **1386**: p. 22-30.

120. McLafferty, F.W., D.A. Stauffer, S.Y. Loh, and C. Wesdemiotis, *Unknown identification using reference mass spectra. quality evaluation of databases.* Journal of the American Society for Mass Spectrometry, 1999. **10**(12): p. 1229-1240.

121. Dromey, R.G., M.J. Stefik, T.C. Rindfleisch, and A.M. Duffield, *Extraction of mass spectra free of background and neighboring component contributions from gas chromatography/mass spectrometry data.* Analytical Chemistry, 1976. **48**(9): p. 1368-1375.

122. Rosenberg, E., V. Kmetov, and M. Grasserbauer, *Investigating the potential of high-performance liquid chromatography with atmospheric pressure chemical ionization-mass spectrometry as an alternative method for the speciation analysis of organotin compounds.* Fresenius' journal of analytical chemistry, 2000. **366**(4): p. 400-407.

123. Covaci, A., S. Voorspoels, L. Ramos, H. Neels, and R. Blust, *Recent developments in the analysis of brominated flame retardants and brominated natural compounds.* Journal of Chromatography A, 2007. **1153**(1): p. 145-171.

124. Keller, R. and R. Topfl, *Process for simultaneously providing synthetic textile materials with an antistatic and dirt-repellent finish*, 1979, Google Patents.

125. Kanikkannan, N. and M. Singh, *Skin permeation enhancement effect and skin irritation of saturated fatty alcohols.* International Journal of Pharmaceutics, 2002. **248**(1–2): p. 219-228.

126. Ryberg, K., M. Isaksson, B. Gruvberger, M. Hindsén, E. Zimerson, and M. Bruze, *Contact allergy to textile dyes in southern Sweden.* Contact Dermatitis, 2006. **54**(6): p. 313-321.