Organohalogen contaminants in wildlife from the Yangtze River Delta

Development of methods and assessments of legacy and emerging persistent organic pollutants

Ge Yin
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Ge Yin
To Yihui, my parents, and aunt.
Cover page: A fictitious football match between organohalogen contaminants and wildlife held in the Yangtze River Delta (Designed by Yihui Zhou and Ziyu Zheng)

Team in left side: Organohalogen contaminants  
Goalkeeper: chlorinated paraffin  
Defender: BDE-47, CB-153, 4,4'-dicofol, 2'-OH-CDE-206  
Midfield: HCB, HCH, PCP  
Forward: OCDD, 4,4'-DDE, HBCDD

Team in right side: Wildlife  
Goalkeeper: cod  
Defender: clam, snail, mussel  
Midfield: toad, eel, snake, frog  
Forward: heron, falcon, tern
List of publications

The thesis is based on the following articles and manuscripts, referred to in the text by their roman numerals (I-VI). Published articles are reprinted with the permission from the publisher.

**Paper I. Chlorinated and brominated organic pollutants in shellfish from the Yellow Sea and East China Sea**


**Paper II. A refined method for analysis of 4,4’-dicofol and 4,4’-dichlorobenzophenone**

Yin, G.; Athanassiadis, I.; Bergman, Å.; Zhou, Y.H.; Qiu, Y.L.; Asplund, L. *Manuscript*

**Paper III. Extensive organohalogen contamination in wildlife from a site in the Yangtze River Delta**


**Paper IV. A novel pollution pattern: Highly chlorinated biphenyls retained in Black-crowned night heron (Nycticorax nycticorax) and Whiskered tern (Chlidonias hybrida) from the Yangtze River Delta**


**Paper V. High concentrations of OCDD and related chemicals in heron and tern eggs from Yangtze River Delta indicating PCP origin**

Zhou, Y.H.; Yin, G.; Asplund, L.; Stewart, K.; Ruokojärvi, P.; Bignert, A.; Rantakokko, P.; Kiviranta, H.; Qiu, Y.L.; Bergman, Å. *Submitted manuscript*
Paper VI. Sampling designs for contaminant temporal trend analyses using sedentary species exemplified by the snails Bellamya aeruginosa and Viviparus viviparus


Contribution to Paper I-VI

Paper I and II: I participated in the design of the study. I was responsible for all the experimental work, data evaluation and I took the lead in writing the manuscript and finalizing the publication.

Paper III: I assisted the experiment, data evaluation and participated in writing.

Paper IV and V: I assisted the experiment, data evaluation and took the lead in writing.

Paper VI: I participated in the design of the study and sampling. I was responsible for the experimental work, assisted data evaluation and participated in writing.

Publication/Manuscripts not included in the thesis

Spatial distribution and Bioaccumulation of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in snails (Bellamya aeruginosa) and sediments from Taihu lake area, China

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## Abbreviations

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<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BFRs</td>
<td>brominated flame retardants</td>
</tr>
<tr>
<td>CPs</td>
<td>chlorinated paraffins</td>
</tr>
<tr>
<td>DCBP</td>
<td>dichlorobenzophenone</td>
</tr>
<tr>
<td>DDT</td>
<td>dichlorodiphenyltrichloroethane</td>
</tr>
<tr>
<td>4,4'-DDT</td>
<td>1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane</td>
</tr>
<tr>
<td>4,4'-DDE</td>
<td>1,1-bis(4-chlorophenyl)-2,2-dichloroethene</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>ECD</td>
<td>electron capture detector</td>
</tr>
<tr>
<td>ECNI</td>
<td>electron capture negative ionization</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact ionization</td>
</tr>
<tr>
<td>GC</td>
<td>gas chromatography</td>
</tr>
<tr>
<td>GPC</td>
<td>gel permeation chromatography</td>
</tr>
<tr>
<td>HBCD</td>
<td>hexabromocyclododecane</td>
</tr>
<tr>
<td>HCB</td>
<td>hexachlorobenzene</td>
</tr>
<tr>
<td>HCHs</td>
<td>hexachlorocyclohexanes</td>
</tr>
<tr>
<td>HRMS</td>
<td>high resolution mass spectrometry</td>
</tr>
<tr>
<td>LC</td>
<td>liquid chromatography</td>
</tr>
<tr>
<td>LCCPs</td>
<td>long chain chlorinated paraffins</td>
</tr>
<tr>
<td>LOD</td>
<td>limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>limit of quantification</td>
</tr>
<tr>
<td>LRMS</td>
<td>low resolution mass spectrometry</td>
</tr>
<tr>
<td>MCCPs</td>
<td>medium chain chlorinated paraffins</td>
</tr>
<tr>
<td>MeO-PCDEs</td>
<td>methoxylated polychlorinated diphenyl ethers</td>
</tr>
<tr>
<td>Na-PCP</td>
<td>sodium pentachlorophenate</td>
</tr>
<tr>
<td>OCDD</td>
<td>octachlorodibenzo-(p)-dioxin</td>
</tr>
<tr>
<td>OCPs</td>
<td>organochlorine pesticides</td>
</tr>
<tr>
<td>OHCs</td>
<td>organohalogen contaminants</td>
</tr>
<tr>
<td>OH-PCDEs</td>
<td>hydroxylated polychlorinated diphenyl ethers</td>
</tr>
<tr>
<td>OND-PCBs</td>
<td>highly chlorinated biphenyls (i.e. PCBs with 8-10 chlorine substituents, octaCBs, nonaCBs &amp; decaCB)</td>
</tr>
</tbody>
</table>
| PBDEs        | polybrominated diphenyl ethers (individual PBDEs are presented as “BDE-#”)
<p>| PCA          | principal component analysis |
| PCBs         | polychlorinated biphenyls (individual PCBs are presented as “CB-#”) |</p>
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCP</td>
<td>pentachlorophenol</td>
</tr>
<tr>
<td>PCDDs</td>
<td>polychlorinated dibenzo-(p)-dioxins</td>
</tr>
<tr>
<td>PCDEs</td>
<td>polychlorinated diphenyl ethers (individual PCDEs are presented as “CDE-#”)</td>
</tr>
<tr>
<td>PCDFs</td>
<td>polychlorinated dibenzofurans</td>
</tr>
<tr>
<td>PFOS</td>
<td>perfluorooctane sulfonate</td>
</tr>
<tr>
<td>POPs</td>
<td>persistent organic pollutants</td>
</tr>
<tr>
<td>SC</td>
<td>Stockholm Convention</td>
</tr>
<tr>
<td>SCCPs</td>
<td>short chain chlorinated paraffins</td>
</tr>
<tr>
<td>SIM</td>
<td>selected ion monitoring</td>
</tr>
<tr>
<td>TCDD</td>
<td>2,3,7,8-tetrachlorodibenzo-(p)-dioxin</td>
</tr>
<tr>
<td>YRD</td>
<td>Yangtze River Delta</td>
</tr>
</tbody>
</table>
1. Background

The development of human civilization is in part related to innovations in development and applications of man-made chemicals. In China, people are proud of the four great innovations: Paper making, Printing, Gunpowder and the Compass. In three of these, chemistry and use of chemicals play a key role. In particular, papermaking and printing technique not only have an invaluable influence to the world, but also make it possible for you to read my thesis from the printed book. However, each coin has two sides. Paper making is the cause of some severe environmental contamination, particularly when chlorine gas came into use for paper pulp bleaching.¹ This type of bleaching led to discharges of a large number of organochlorine contaminants, including poly-chlorinated dibenzo-\(p\)-dioxins (PCDDs) and dibenzofurans (PCDFs).² However, a large number of other sources have emerged over the years as the origin of organohalogen contaminants (OHCs). First to be discovered were those intentionally distributed in the environment, pesticides; soon followed by intentionally produced but unintentionally distributed in the environment such as the polychlorinated biphenyls (PCBs).

One of the most well-known OHCs is dichlorodiphenyltrichloroethane (DDT). It was first synthesized in 1874 by Othmar Zeidler.³ However, it was not until the 1930’s as Paul Hermann Müller discovered DDT’s insecticidal properties that it really became a very important innovation. DDT was applied in a lot of applications and deliberately used for insect control. DDT was even applied in soldiers helmets to protect them from vector transferred diseases during the Second World War.⁴ Müller was awarded the Nobel Prize in Physiology or Medicine in 1948 but already at this time a warning had been raised for use of DDT.⁵ The successful application of DDT inspired the development of new organic pesticides and other man-made chemicals. In 1962, Rachel Carson published the book “Silent Spring”, elaborating how pesticides are related to adverse effects in the ecosystems.⁶ This book caught enormous attention both from scientific and public side and has been considered as one of the most influential book on environmental pollution in the last century.
From the mid 1960’s, more research on environmental pollution was initiated and details of the ingredients in DDT were discovered. It was shown that the main ingredient in DDT was 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (4,4’-DDT), which is metabolized to 1,1-bis(4-chlorophenyl)-2,2-dichloroethene (4,4’-DDE), but also a full range of other metabolites. DDT, and its main metabolites DDE, showed negative effects such as eggshell thinning in birds of prey. DDT is still today one of the major environmental pollutants threatening both wildlife and human health.

Severe contamination in wildlife and an accident in Japan led to the identification of PCBs as an environmental contaminant and a class of chemicals of great concern in relation to health. The important discoveries were the identification of PCB in white-tailed sea eagle in Sweden and the contamination of rice oil from a factory in Southern Japan, resulting in thousands of people being poisoned.

Another astonishing example of environmental pollution is the discovery of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure coming from impurities in Agent Orange which was used as a defoliant during the war in Vietnam. This is only one example of how unintentional byproducts in a chemical process are unintentionally distributed in the environment. The class of PCDD/Fs, commonly referred to as “dioxins” led to intensive research from the latter part of the 1970’s due to their toxicity and common formation from numerous industrial processes.

Other technically produced chemicals, such as polybrominated diphenyl ethers (PBDEs) and per- and polyfluorinated substances, in particular represented by perfluorooctane sulfonate (PFOS) came into focus during 1980’s/1990’s and in the beginning of the new century, respectively. Although the usage and sources are different, these chemicals mentioned above (DDTs, PCBs, PCDD/Fs, PBDEs and PFOS) have been considered to share overall similar properties. Their structures make them all persistent in the environment; their lipophilic properties make the first four bioaccumulative, while the proteinophilic character of PFOS make this compound accumulate in blood and liver; their semi-volatility make them undergo long range environmental transportation and finally their toxicity cause wide attention. In 2001, the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs) was signed and has been ratified by 172 countries in the world since 2004. This is an international treaty to protect human health and the environment from POPs. It can be achieved by elimination, restriction and/or controlling the production process or
finding the substitution method. Initially there were 12 groups of chemicals listed in the SC and today the number has expanded to 26 (Table 1.1). However, it must not be overlooked that other OHCs, not currently listed as POPs according to the SC, the so called “legacy POPs”, have a potential to fulfill the requirements as new POPs. In this thesis these chemicals are divided into three categories. The first category includes impurities and byproducts from the POPs and OHCs in general (e.g. polychlorinated diphenyl ethers (PCDEs), hydroxylated polychlorinated diphenyl ethers (OH-PCDEs)). The second category is chemicals which are produced for substitution of POPs, such as chlorinated paraffins (CPs) and dicofol. The third category is the novel pattern and profile of POPs (e.g. highly chlorinated biphenyls, the octa-, nona- and decaCBs (OND-PCBs)) as well as novel OHCs. Due to the limited study of these categories compared with POPs, they are considered as emerging environmental contaminants in this thesis. It means that these contaminants have been documented regarding production and use and/or have been shown to occur in wildlife and humans.

China, with a population of 1.4 billion, is the biggest developing country in the world. Studies on environmental exposure of OHCs are highly necessary due to, but not limited to, the following reasons: (1) Agriculture is considered as the lifeline to afford such a large population. Therefore, organochlorine pesticides (OCPs) and antimicrobial agent are widely used to protect crops from pests and farmed animal; (2) OCPs have been used for disease prevention (e.g. malaria, schistosomasis); (3) the rapid industrialization and urbanization has been taken place during the past 40 years. Chemicals are produced not only for domestic use but also for export; (4) certain hotspots of electron waste recycling have been reported regarding legacy POPs, resulting in extremely high level of contamination levels of PBDEs calling for concern on public health and food safety issue to local residents.

To date, numerous studies have been conducted on OHCs level in the environment in China, as exemplified with a few references. However, the studies which focus on emerging environmental contaminants are less frequent than those on POPs. The studies pursued are not geographically evenly distributed, as much work has been carried out in South China (Pearl River Delta) and North China, whereas information on environmental exposure level on OHCs in the Yangtze River Delta (YRD) are more limited. In terms of environmentally relevant studies performed in the YRD, most of them have been conducted in abiotic matrices.
Table 1.1. Basic information on legacy POPs under the Stockholm Convention, with those included in this thesis are marked with an asterisk (*).

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Use/Source</th>
<th>Annex</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2001</td>
</tr>
<tr>
<td>Chlordane</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2001</td>
</tr>
<tr>
<td>Chlordecone</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2009</td>
</tr>
<tr>
<td>Commercial octa-bromo diphenyl ethers*</td>
<td>PBDEs</td>
<td>Industrial chemical</td>
<td>A</td>
<td>2009</td>
</tr>
<tr>
<td>Commercial penta-bromo diphenyl ethers*</td>
<td>PBDEs</td>
<td>Industrial chemical</td>
<td>A</td>
<td>2009</td>
</tr>
<tr>
<td>DDT*</td>
<td></td>
<td>Pesticide</td>
<td>B</td>
<td>2001</td>
</tr>
<tr>
<td>Dieldrin</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2001</td>
</tr>
<tr>
<td>Endrin</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2001</td>
</tr>
<tr>
<td>Endosulfan</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2011</td>
</tr>
<tr>
<td>Heptachlor</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2001</td>
</tr>
<tr>
<td>Hexabromobiphenyl</td>
<td>HBB</td>
<td>Industrial chemical</td>
<td>A</td>
<td>2009</td>
</tr>
<tr>
<td>Hexabromocyclododecane*</td>
<td>HBCDD</td>
<td>Industrial chemical</td>
<td>A</td>
<td>2013</td>
</tr>
<tr>
<td>Hexachlorobenzene*</td>
<td>HCB</td>
<td>Pesticide/Industrial chemical/Byproduct</td>
<td>A/C</td>
<td>2001</td>
</tr>
<tr>
<td>Hexachlorobutadiene</td>
<td></td>
<td>Industrial chemical</td>
<td>A</td>
<td>2015</td>
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<tr>
<td>α-hexachlorocyclohexane*</td>
<td>α-HCH</td>
<td>Pesticide</td>
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<td>β-hexachlorocyclohexane*</td>
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<td>Pesticide</td>
<td>A</td>
<td>2009</td>
</tr>
<tr>
<td>Lindane*</td>
<td></td>
<td>γ-HCH</td>
<td>A</td>
<td>2009</td>
</tr>
<tr>
<td>Mirex*</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2001</td>
</tr>
<tr>
<td>Pentachlorobenzene</td>
<td>PeCBz</td>
<td>Pesticide/Industrial chemical/Byproduct</td>
<td>A/C</td>
<td>2009</td>
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<td>Pentachlorophenol and its salts and esters*</td>
<td>PCP</td>
<td>Pesticide</td>
<td>A</td>
<td>2015</td>
</tr>
<tr>
<td>Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyle fluoride</td>
<td>PFOS</td>
<td>Industrial chemical</td>
<td>B</td>
<td>2009</td>
</tr>
<tr>
<td>Polychlorinated biphenyls*</td>
<td>PCBs</td>
<td>Industrial chemical/Byproduct</td>
<td>A/C</td>
<td>2001</td>
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<tr>
<td>Polychlorinated naphthalenes</td>
<td>PCNs</td>
<td>Industrial chemical/Byproduct</td>
<td>A/C</td>
<td>2015</td>
</tr>
<tr>
<td>Polychlorinated dibenzo-p-dioxins*</td>
<td>PCDDs</td>
<td>Byproduct</td>
<td>C</td>
<td>2001</td>
</tr>
<tr>
<td>Polychlorinated dibenzofurans*</td>
<td>PCDFs</td>
<td>Byproduct</td>
<td>C</td>
<td>2001</td>
</tr>
<tr>
<td>Toxaphene</td>
<td></td>
<td>Pesticide</td>
<td>A</td>
<td>2001</td>
</tr>
</tbody>
</table>

a. Annex A: Elimination; Annex B: restriction; Annex C: unintentional production
1.1 Aim of thesis

This thesis includes both chemical exposure assessments and method development. Paper I, III, IV and V aimed to increase the collective knowledge on OHCs in several wildlife species to improve the knowledge of the pollution burden of OHCs in the Yangtze River Delta. To reach this goal, both quantitative and qualitative analyses have been applied. Paper II is about methodological development for a specific compound, dicofol, which has been listed as a candidate under the SC. Paper VI is to design sampling strategy by taking advantage of power analysis. The aims of the individual papers I - VI are summarized as follows:

Paper I: The aim was to determine residual concentrations of OCPs, PCBs, brominated flame retardants (BFRs) in shellfish from a few locations along the eastern coastline of China. In addition, the paper described a general contamination situation in YRD by using shellfish as a bioindicator.

Paper II: Extremely high level of 4,4’-DDE found in Paper I arose our interests to focus on DDT related compounds. DDT was banned for agricultural use from 1983 and has mainly been used for dicofol production since then. However, the environmental exposure data on dicofol are very limited probably due to the analytical challenge. Hence, the aim of Paper II was to establish an analytical method for dicofol and dichlorobenzophenone (DCBP) analyses. DCBP is the main decomposition product of dicofol in environment and during instrumental analysis.

Paper III: The aim was to screen for OHCs in six wildlife species in a paddy field site near a chemical industry park in the YRD and to quantify selected OHCs in order to establish a baseline for future environmental monitoring program.

Paper IV: The screening results from Paper III showed that birds (e.g. peregrine falcon and Chinese pond heron) is promising for investigation of OHCs pollution probably due to bioaccumulation and biomagnification effects. Hence, bird eggs were used for a step forward study. The aim in Paper IV was to measure the OCPs, PCBs and PBDEs level in two water-living bird species egg, and to compare these levels with bird eggs from other part of world to assess the relevant contamination situation in YRD.

Paper V: The aim was to use the same matrices as in Paper IV, but to focus on PCDD/Fs and related compounds, i.e. PCDEs, OH-PCDEs,
methoxylated polychlorinated diphenyl ethers (MeO-PCDEs) and to discuss their relation to pentachlorophenol (PCP).

Paper VI: The main objective is to explore the consequences, in terms of statistical power, using different sampling strategies to detect future temporal trends of contaminants level in sedentary species.
2. Introduction

2.1 Yangtze River Delta

The Yangtze River Delta is situated in the downstream of the Yangtze River, which is the third longest river in the world with a total length of 6300 kilometers, originated on the Qinghai Tibet Plateau. There are different definitions of YRD. From geographic point of view, YRD encompasses south part of Jiangsu Province, Shanghai Municipality and north part of Zhejiang Province. From economic zone point of view, YRD encompasses the whole Jiangsu Province, Shanghai Municipality and Zhejiang Province (Figure 2.1).

YRD has a marine monsoon subtropical climate, with hot and humid summers, warm spring and autumn, and cold and dry winter. Historically, YRD is famous for its beautiful scenery and outstanding people. Bai Juyi (白居易, 772-846), who was one of the household poet living in the Tang Dynasty (618-907) created popular poetries (忆江南) to depict the beautiful scenery written as follows:

江南好，风景旧曾谙；日出江花红胜火，春来江水绿如蓝。能不忆江南？
江南忆，最忆是杭州；山寺月中寻桂子，郡亭枕上看潮头。何日更重游？
江南忆，其次忆吴宫；吴酒一杯春竹叶，吴娃双舞醉芙蓉。早晚复相逢!
Today, YRD is one of the most dense metropolitan areas in the world including a large number of cites with multimillions of inhabitants. YRD is an important area of economy in China, which contributed up to 19% of the Chinese Gross Domestic Production in 2014. YRD contains highly fertile soil. YRD is dotted with more than 250 lakes with abundant water and fish resources, and Zhoushan fishing ground, located in north-east of Zhejiang Province, is the biggest fish ground in China. Therefore, YRD has been called “the town of rice and fish” in China. As a result, the use of pesticides and veterinary drugs in farming and aquaculture is extensive. The industrial activity in YRD is diverse, including refineries, steel factories, electronic waste recycling and numerous chemical manufacturing plants such as production of fine chemicals, textile, pesticides and pharmaceuticals.

The rapid economic growth has been accompanied by environmental pollution. Air pollution is a critical issue caused by e.g. vehicle exhaust, petroleum industrialization and coal burning. Eutrophication frequently occurs in lakes as a response to increased levels of nutrients, resulting in
The water is also polluted by organic and inorganic compounds e.g. POPs, heavy metals and antibiotics. The intensive application of pesticides to the crops leads to the soil contamination. All of these imply increasing risk to ecosystem and human health in the YRD.

### 2.2 Legacy POPs

Table 1.1 is presenting all chemicals included in the Stockholm Convention on persistent organic pollutants, legacy POPs. In total, there are 26 POPs in the SC today. The POPs are subdivided according to the Annexes A-C into those to be eliminated, restricted and unintentionally produced, respectively. The POPs assessed in the present thesis are introduced below while those not studied are left out in this context. The POPs studied are sub-sectioned into those: i) intentionally produced and distributed to the environment; ii) intentionally produced and unintentionally distributed to the environment and iii) unintentionally produced and released to the environment. The chemical structures of POPs included in this thesis are shown in Figure 2.2.

![Chemical structures of the POPs addressed in this thesis.](image)

**Figure 2.2.** Chemical structures of the POPs addressed in this thesis.
2.2.1 Chemicals intentionally produced and distributed to the environment

The work in this thesis includes chemical analysis of DDT, hexachlorocyclohexanes (HCHs), Mirex and PCP. The text below focuses on information, relevant for China (c.f. Table 2.1), on the chemicals discussed in this thesis.

**DDTs**

DDT was introduced as an example of POPs described in Chapter 1, above. In China, DDT was mainly used to control malaria from 1950s to 1983. The total production of DDT during that period of time was about 0.46 million tons, which accounted for 20% of the global production. The technical DDT product is composed of 4,4'-DDT (80%) and 2,4'-DDT (15-20%). In the environment, 4,4'-DDT is readily transformed to 4,4'-DDE and 4,4'-DDD. The ratio between 4,4'-DDT and ∑4,4'-DDTs (sum of 4,4'-DDT, 4,4'-DDE and 4,4'-DDD) are commonly used to assess whether there is a new input of DDT in the environment, or not.

Even though DDT was prohibited for agriculture use for more than 30 years, in China, the residue of DDTs remains high in the Chinese environment. DDTs are determined as the predominant OCPs in shellfish (Paper I), paddy field species (Paper III) and water-living bird eggs (Paper IV). This may be attributed to (1) historic application of DDT; (2) use of DDT outside the agricultural sector such as in hull paints for boats; (3) illegal use of DDT and (4) as a starting material for production of dicofol. It was estimated that almost 9000 tons of DDT and related compounds were released to the environment from dicofol production, during 1988-2002. More information on dicofol is given in Chapter 2.3, below.

**Hexachlorocyclohexanes**

HCHs were used as pesticides in agriculture. α-, β- and γ-HCH are the main isomers in the products. There are two types of HCHs widely produced. One is the technical HCHs (65-70% α-HCH, 7-10% β-HCH and 15% γ-HCH) and the other is Lindane (99% γ-HCH). γ-HCH has the highest insecticidal potency whereas β-HCH is the most persistent and bioaccumulative isomer. All of these three isomers added into POPs list under SC in 2009. From 1950s to 1983, about 4.9 million tons of technical HCH was produced mainly for agricultural use in China.
though the technical HCHs have been banned since 1983, Lindane has been produced and used in China since 1991 until being prohibited in 2004.\textsuperscript{39} Concentrations of HCHs are reported in shellfish (Paper I), paddy field species (Paper III) and water-living bird eggs (Paper IV), in this thesis.

\textbf{Table 2.1. Basic situation of selective OHCs in China.}\textsuperscript{40}

<table>
<thead>
<tr>
<th>Type</th>
<th>Output (tons/year)</th>
<th>Number of production enterprises</th>
<th>Accumulated output (tons)</th>
<th>Main uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT\textsuperscript{a}</td>
<td>3 900</td>
<td>2 (11)</td>
<td>464 000</td>
<td>Malaria control</td>
</tr>
<tr>
<td>HCHs</td>
<td></td>
<td></td>
<td>4 900 000</td>
<td>Insecticide</td>
</tr>
<tr>
<td>Mirex\textsuperscript{a}</td>
<td>15</td>
<td>3 (7)</td>
<td>160</td>
<td>Termite elimination and control</td>
</tr>
<tr>
<td>HCB\textsuperscript{a}</td>
<td></td>
<td></td>
<td>79 000</td>
<td>PCP raw material</td>
</tr>
<tr>
<td>PCBs\textsuperscript{a}</td>
<td></td>
<td>(4)</td>
<td>7 000-10 000</td>
<td>In transformers &amp; capacitors</td>
</tr>
<tr>
<td>PBDEs\textsuperscript{b}</td>
<td>44 000</td>
<td>(&gt;100)</td>
<td></td>
<td>Flame retardant</td>
</tr>
<tr>
<td>HBCDD\textsuperscript{b}</td>
<td>7 500</td>
<td>(&gt;100)</td>
<td></td>
<td>Flame retardant</td>
</tr>
<tr>
<td>Dicofol\textsuperscript{c}</td>
<td>530</td>
<td>3-5</td>
<td>19 500</td>
<td>Acaricide</td>
</tr>
<tr>
<td>CPs\textsuperscript{d}</td>
<td>1 000 000</td>
<td>&gt;100</td>
<td></td>
<td>Flame retardant</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Number of production enterprises in 2004. In parentheses shows the total number of production enterprises historically; \textsuperscript{b} Production in 2006, over 100 factories manufactured flame retardants in China until 2009\textsuperscript{41}; \textsuperscript{c} output in 2012, accumulated output during 2000-2012\textsuperscript{42}; \textsuperscript{d} Production in 2013.\textsuperscript{43}

\textit{Mirex}

Mirex was used as a pesticide to control fire ants in North America during the 1960s-1970s.\textsuperscript{44} It has also been applied as a flame retardant under the trade name Dechlorane in rubber, paint, plastics and electronic equipment.\textsuperscript{45} Mirex has been replaced by other Dechlorane series compounds (Dec 602-605).\textsuperscript{45, 46} In China, Mirex was primarily produced as a pesticides in order to control termites from the 1960s.\textsuperscript{46} It was prohibited in the early 1980s. However, due to the lack of cost-effective pesticides to replace Mirex in South China, Mirex was reused until 2009.\textsuperscript{47} The accumulated production of Mirex was estimated to be about 160 tonnes until 2004.\textsuperscript{40} Wang and co-workers detected Mirex levels in soil in
Liyang city, which was a Mirex manufacturing base and located in Jiangsu province, with a concentration of 2.9 pg - 4.3 ng g\(^{-1}\) dw in soil samples.\(^{47}\) Mirex is identified and reported in paddy field species (Paper III) and quantified in shellfish (Paper I) and bird eggs (Paper IV).

**Pentachlorophenol**

PCP is one of the most widely used fungicides for wood preservation and as a pesticide for Schistosomiasis vector control in China.\(^{48}\) The physico-chemical properties of PCP are different from other POPs since this compound is a proteinophilic pollutant. PCP is more volatile and water soluble than other POPs. It was not until 2015 that PCP was included in the SC even though it has a long production and use history.\(^{17}\) PCP and sodium pentachlorophenate (Na-PCP) are widely used in China, especially in southern area across the Yangtze River watershed from the mid-1950s, to protect people from the *Schistosomiasis japonica* threat. This disease killed millions of people.\(^{49}\) However, PCP has been related to many adverse effects on human health, such as thyroid disrupting effects\(^{50}\) and of being carcinogenic.\(^{51}\) In 1997, China once restricted the production and use of Na-PCP for its potential health risk.\(^{49}\) However, with the re-emergence of schistosomiasis in the traditional epidemic areas,\(^{52}\) the production and use of PCP has increased again. The annual national output reached approximately 10 000 tonnes of PCP in 1997, which was approximately 20% of global production. In 2006, about 60% of the PCP and Na-PCP production were applied to kill schistosomiasis intermediate host snails; while wood preservation and other uses accounted for the other 40% in China.\(^{53}\)

![Figure 2.3. Pathway for formation of octachlorodibenzo-*p*-dioxin (OCDD) from pentachlorophenol (PCP).](image)

PCP is a source of the octachlorodibenzo-*p*-dioxin (OCDD, Figure 2.3).\(^{54}\) PCP can also be transformed to pentachloroanisole, especially in aerobic
soils. Other biotransformation pathways for PCP include acetylation, dechlorination and hydroxylation.

Concentrations of PCP and pentachloroanisole were assessed in bird eggs as described in Paper V. Pentachloroanisole has also been detected in snails sampled in Tianmu lake (unpublished data).

2.2.2 Chemicals intentionally produced and unintentionally distributed to the environment

Polychlorinated biphenyls

PCBs were extensively used as heat exchange fluids, additive in paint, sealants and in electric transformers and capacitors since 1930s. All main production was however stopped during the 1970s-1980s. In China, about 7 000-10 000 tons of PCBs were produced during 1965 to 1974. The two main PCB products used in China were similar to Aroclor 1242 and Aroclor 1254, respectively. Detailed data on the composition of Aroclor products are given in the Appendix, Table A1. In addition, PCBs was imported to China from Europe without being informed in the 1950s-1980s. Moreover, PCBs has also been demonstrated to be generated during the combustion of chlorinated chemicals.

Theoretically, there is a number of 209 PCB congeners for which Ballschmitter and co-workers developed a numbering system. The abbreviation system has been acknowledged by the International Union of Pure and Applied Chemistry (see Appendix, Table A2). This abbreviation system has been adopted for applications to other chemical classes such as PBDEs and PCDEs.

Among the 209 PCB congeners, CB-153 is commonly reported as the most abundant congener and may accordingly be used as an indicator for PCBs contamination. Xing and co-workers overviewed PCBs pollution in China and concluded that PCB levels in environmental media were relatively low on a national basis.

The concentrations and congener profile of PCBs are reported within this thesis, in shellfish (Paper I), paddy field species (Paper III) and bird eggs (Paper IV). Occasionally, a novel pattern of PCBs (with chlorine number of 8-10) were identified in Chinese pond heron (Paper III, c.f. Chapter 2.3).
**Polybrominated diphenyl ethers**

PBDEs is the most well-known BFR and have been widely used in polymers for fire safety in e.g. furniture, textiles and electronic since 1970s.\(^{61-64}\) PentaBDE, OctaBDE and DecaBDE are the three major commercial products once on the market (see Appendix, Table A3).\(^{65}\) The naming system of the 209 theoretical PBDE congeners is consistent with that of the PCB congeners (Table A2).

Several studies have indicated associations between PBDEs and effects on e.g. the thyroid, neurobehavioral development and reproduction.\(^{66,67}\) While PentaBDE and OctaBDE are included among the POPs\(^{17}\), DecaBDE is not. However, DecaBDE is in the reviewing process for inclusion among the POPs.\(^{68}\) China officially prohibited the production and usage of Penta- and OctaBDE in 2006.\(^{19}\) However, DecaBDE may still be produced in China whereas it was phased out in North America and Europe by 2013.\(^{68}\) The annual production of DecaBDE in China was about 15 000 tons in 2006.\(^{19}\) In addition, some cities in the south of China are the dominating e-waste recycling sites in the world.\(^{21,69}\) High contamination levels of BFRs have been reported in both abiotic and biotic environmental samples in these sites.\(^{19,69,70}\)

PBDEs are commonly metabolized through oxidative transformations and via reductive debromination.\(^{71}\) The hydroxylation is induced by cytochrome P450 enzymes and mostly takes place in meta- and/or para-position of the ring with few exception (e.g. 6-OH-BDE-47).\(^{72}\) However, OH-PBDEs are also formed via natural processes forming primarily PBDEs with the OH- group substituted in ortho-position.\(^{73}\) PBDEs may also undergo reductive debrominations under abiotic conditions.\(^{74,75}\)

Concentrations of PBDEs are presented within this thesis, in shellfish (Paper I), paddy field species (Paper III) and bird eggs (Paper IV). In Paper VI, PBDEs are analyzed in snail samples individually and those results were used for computer simulation.

**Hexabromocyclododecane**

Hexabromocyclododecane (HBCDD) is one of the most common BFRs, applied in polystyrene foam, textile and electronic equipment.\(^{76}\) HBCDD was first identified in fish and sediment from river Viskan in Sweden.\(^{77}\) Commercial HBCDD products mainly consist of \(\alpha-, \beta-\) and \(\gamma-\)isomers, with \(\alpha\)-HBCDD predominating in biological samples.\(^{78}\) HBCDD, DecaBDE and tetrabromobisphenol A (TBBPA) were for long, and globally the
dominating BFRs. HBCDD was recently added into POPs under SC. The historic use of HBCDD in China is unknown. However, the levels of HBCDD in northern hemisphere are much higher than those in southern hemisphere. The environmental level of HBCDD in European and Asian environment has been reviewed by Law et al.

Comparisons of concentrations on HBCDD with PBDEs are presented, in this thesis, in shellfish (Paper I), paddy field species (Paper III) and bird eggs (Paper IV).

Hexachlorobenzene

Hexachlorobenzene (HCB) can be generated from many sources, such as production and use as a pesticide, byproduct from manufacturing of other chemicals (e.g. organic pigment), recycling and combustion. In China, HCB has never, to my knowledge, been used directly as a pesticide. The main use includes production of PCP and its salt by reaction between HCB and e.g. sodium or potassium hydroxide. Other applications of HCB include use as solvent reagent. It was reported that >95% of HCB was used to produce PCP/Na-PCP during 1988-2003 (Figure 2.4), with an accumulative amount of 79,000 tons. In general, HCB levels in environmental compartments in China are in the moderate range when compared on a global scale.

In this thesis, Paper I and III, HCB is reported together with PCB and OCP levels, while in Paper V, HCB is reported together with occurrence of PCP and its related contaminants.

![Figure 2.4. Route for PCP and PCP-Na production.][a] alkali hydrolysis; [b] acidification.

2.2.3 Unintentionally produced POPs

PCDD/Fs, commonly referred to as “dioxins” consisting of a total of 75 PCDDs and 135 PCDFs congeners. Particularly a subset of the PCDDs/Fs
are highly toxic.\textsuperscript{13} Among all 210 PCDD/F congeners, the main concern is related to seventeen 2,3,7,8-substituted congeners. In order to assess the risk of PCDD/Fs, Toxicity Equivalency Factors (TEF) was developed to quantify the toxicity of 2,3,7,8-substituted PCDD/Fs.\textsuperscript{83, 84} This concept is set based on two assumptions. Firstly, all of the seventeen PCDD/F congeners share common mechanisms. One of the major mechanisms of PCDD/Fs toxicity is that these contaminants bind to the aryl hydrocarbon receptor (Ah-receptor), and further bind to a specific regions of the DNA chain.\textsuperscript{84} Secondly, the toxicity effects are additive, rather than synergistic or antagonistic.

Table 2.2. Toxic PCDD/F congeners patterns and relative contribution from house coal burning (HB)\textsuperscript{85}, gasous emission (GE)\textsuperscript{86}, waste water from pulp mills (WPW)\textsuperscript{87, 88}, Agricultural straw open burning (SOB)\textsuperscript{89}, atmospheric deposition (AD)\textsuperscript{90, 91}, ink printing (IP)\textsuperscript{87}, chloronitrofen (CNP)\textsuperscript{92} and pentachlorophenol (PCP)\textsuperscript{86, 87, 92}

<table>
<thead>
<tr>
<th>Congener Pattern</th>
<th>HB</th>
<th>GE</th>
<th>WPW</th>
<th>SOB</th>
<th>AD</th>
<th>IP</th>
<th>CNP</th>
<th>PCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,3,7,8-Tetra-CDD</td>
<td>1%</td>
<td>2%</td>
<td>1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,7,8-Penta-CDD</td>
<td>1%</td>
<td>2%</td>
<td>-</td>
<td>1%</td>
<td>1%</td>
<td>-</td>
<td>2%</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,4,7,8-Hexa-CDD</td>
<td>-</td>
<td>1%</td>
<td>-</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>1,2,3,6,7,8-Hexa-CDD</td>
<td>1%</td>
<td>2%</td>
<td>5%</td>
<td>2%</td>
<td>1%</td>
<td>4%</td>
<td>36%</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,7,8,9-Hexa-CDD</td>
<td>-</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>1%</td>
<td>2%</td>
<td>14%</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-Hepta-CDD</td>
<td>7%</td>
<td>5%</td>
<td>19%</td>
<td>11%</td>
<td>11%</td>
<td>29%</td>
<td>7%</td>
<td>12%</td>
</tr>
<tr>
<td>OCDD</td>
<td>58%</td>
<td>4%</td>
<td>45%</td>
<td>15%</td>
<td>55%</td>
<td>44%</td>
<td>1%</td>
<td>66%</td>
</tr>
<tr>
<td>2,3,7,8-Tetra-CDF</td>
<td>16%</td>
<td>7%</td>
<td>15%</td>
<td>3%</td>
<td>1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,7,8-Penta-CDF</td>
<td>5%</td>
<td>5%</td>
<td>1%</td>
<td>4%</td>
<td>1%</td>
<td>-</td>
<td>1%</td>
<td>-</td>
</tr>
<tr>
<td>2,3,4,7,8-Penta-CDF</td>
<td>3%</td>
<td>18%</td>
<td>-</td>
<td>6%</td>
<td>1%</td>
<td>-</td>
<td>1%</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,4,7,8-Hexa-CDF</td>
<td>1%</td>
<td>8%</td>
<td>4%</td>
<td>6%</td>
<td>1%</td>
<td>1%</td>
<td>-</td>
<td>1%</td>
</tr>
<tr>
<td>1,2,3,6,7,8-Hexa-CDF</td>
<td>1%</td>
<td>8%</td>
<td>1%</td>
<td>6%</td>
<td>2%</td>
<td>-</td>
<td>2%</td>
<td>-</td>
</tr>
<tr>
<td>2,3,4,6,7,8-Hexa-CDF</td>
<td>-</td>
<td>9%</td>
<td>1%</td>
<td>1%</td>
<td>4%</td>
<td>-</td>
<td>16%</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,7,8,9-Hexa-CDF</td>
<td>1%</td>
<td>4%</td>
<td>-</td>
<td>7%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1,2,3,4,6,7,8-Hepta-CDF</td>
<td>2%</td>
<td>15%</td>
<td>3%</td>
<td>21%</td>
<td>9%</td>
<td>4%</td>
<td>1%</td>
<td>8%</td>
</tr>
<tr>
<td>1,2,3,4,7,8,9-Hepta-CDF</td>
<td>1%</td>
<td>3%</td>
<td>-</td>
<td>3%</td>
<td>1%</td>
<td>-</td>
<td>-</td>
<td>3%</td>
</tr>
<tr>
<td>OCDF</td>
<td>2%</td>
<td>5%</td>
<td>5%</td>
<td>12%</td>
<td>1%</td>
<td>14%</td>
<td>-</td>
<td>7%</td>
</tr>
</tbody>
</table>
PCDD/Fs have never been considered as marketable products, as e.g. PCBs or PBDEs. Natural sources (e.g. forest fire and volcanic eruptions) have been considered as a minor source for PCDD/Fs in environment compared with anthropogenic sources. Previous studies identified and apportioned a number of PCDD/Fs sources by human activity, including production of chlorinated chemicals (e.g. chlorinated phenols), chlorine bleaching, chlor-alkali production, municipal and medical waste incineration, vehicle exhaust emissions and crop burning (Table 2.2). It is noteworthy that the contamination sources of PCDD/Fs may be dispersed, making source interpretation and identifications complicated.

In Paper V, concentrations of seventeen 2,3,7,8-substituted PCDD/F congeners in eggs from two bird species are reported to add knowledge on dioxin exposure risks in the YRD.

### 2.3 Emerging environmental contaminants

Legislative regulation managed to restrict and prohibit the production and use of what is discussed as legacy POPs, today. In parallel to regulations of certain chemicals goes a strive for appropriate replacements, which in turn call for new studies related to the replacements safety characteristics. In addition, some impurities, byproducts and metabolites among the legacy POPs may still call for further research. Several of these emerging chemicals are structurally related to POPs and also share similar chemical and biological properties as the legacy POPs. These emerging chemicals identified and quantified in individual papers are introduced in this part of the thesis. The chemical structures of the emerging environmental contaminants included in the thesis are shown in Figure 2.5.

**Chlorinated paraffins**

CPs are also known as polychlorinated alkanes, are indeed emerging organic pollutants even though well-known OHCs. Depending on the carbon chain length, CPs are divided into three classes: short chain (C_{10}-C_{13}, SCCPs), medium chain (C_{14}-C_{17}, MCCPs) and long chain CPs (C_{18}-C_{30}, LCCPs). The chlorine content by weight in CPs mixture ranges from 30% to 70%. CPs have been used in a wide range of area such as additive in metal cutting fluids, and applied as plasticizers and flame retardants. China is the by far dominating producer and consumer of CPs in the world. The annual CPs production was about one million tonnes in 2013.
Figure 2.5. Chemical structures, general or specific, of the emerging environmental contaminants addressed in this thesis are presented. From top left to right bottom: Chlorinated paraffins (CPs) are here exemplified with one structure of hexachlorinated dodecane only representing one structure of thousands of CP congeners with different chain lengths (C<sub>10</sub>–C<sub>30</sub>), and chlorine substituents; PCDEs (polychlorinated diphenyl ethers), general structure, of a class of chemicals that theoretically constitute 209 congeners; OH-PCDEs are hydroxylated PCDEs; MeO-PCDEs are methylated PCDEs; OND-PCBs represent octa- to decachlorinated biphenyls; and finally two specific chemical structures: 4,4′-dicofol which is 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethanol and 4,4′-dichlorobenzophenone (DCBP).

The primary product in China is CP-52, contributing to 80% of the total CP production, followed by CP-42 and CP-70. The toxicity studies on CPs have been primarily carried out by commercial products. It was shown that CPs had low acute toxicity to mammals but SCCPs show acute toxicity to freshwater and marine invertebrates. This could be due to the low molecular size and high viscous nature of SCCPs. SCCPs has been detected and reported from sediments in remote areas of Canada, indicating the long range environmental transportation potential. Wang and co-workers studied SCCPs and MCCPs level, pattern in air and soil in the Pearl River Delta, and the authors concluded that shorter-chain CPs with less chlorine substituents, were enriched in air. SCCP are accordingly more likely to undergo transport to the rural areas, while longer-chain with a high chlorine content are concentrated in soil. Many researchers observed that SCCPs can be biomagnified in aquatic food chains. Consequently, SCCP has been proposed as a POPs candidate on Annex E of the SC by 2015.

Due to the number of carbon and substitute position of chlorine in the carbon chain skeleton, CPs have numerous isomers and homologous, which make the CPs quantification become a challenge for environmental and analytical chemists. To date, there is no standard method, but gas
chromatography coupled to a low resolution mass spectrometry (GC-LRMS) is the most commonly used method. Information and challenge on CPs analysis is described in Chapter 3.

The environmental exposure levels of CPs have been summarized in a few review articles. During the past decade, the interests of CPs exposure in China have increased significantly. However, most of the studies are focused on the situation in the Pearl River Delta and north part of China (Yellow Sea, Bohai Sea).

In this thesis (Paper III), severe contamination of CPs is detected in wildlife collected from a paddy field close to an industry park in Shanghai.

*Polychlorinated diphenyl ethers*

PCDEs are a group of chemicals, which structurally resemble the PBDEs, just substituted with chlorine instead of bromine. PCDE congeners are numbered as the PCBs and PBDEs (c.f Appendix Table A2). PCDEs were first detected as impurities in pentachlorophenol, more than 40 years ago. The PCDEs may be formed as outlined in Figure 2.6. It has been detected in a number of biological samples. The first scientific article concerning PCDEs residue in biota was conducted on bivalves from Narrangessett Bay, USA. Tri-CDE and tetraCDE were detected in mussels. However, the majority of PCDEs’ environmental exposure levels were published by Koistinen and co-authors in a set of Finish studies in the 1990s. They reported PCDEs level in salmon, white-tailed sea eagles, guillemots, pike and seal collected near a paper pulp mill. Fifty PCDE congeners were also analyzed in a wood preservative, named Ky-5 used at this site. The results showed that the tetra- to hexaCDEs were the predominant congeners in the wildlife analyzed. In addition to impurities from technical chlorophenols, PCDEs are considered to be formed by incomplete combustion processes. Further, PCDEs were detected in human adipose tissue in Canada, USA and Finland. One study performed on PCDEs level in food in Spain showed that the intake of PCDEs (41 ng/day) was in the same order of magnitude as those of PBDEs (97 ng/day).

Concentrations of PCDEs in bird eggs are presented in Paper V. To our knowledge, this is the first study conducted on PCDEs exposure in wildlife in China.
There is a commercial production of some OH-PCDEs, e.g. 2,4,4'-trichloro-2'-hydroxyl diphenyl ether, known by the trade name triclosan, which is an antibacterial agent used i.e. in personal care products, toothpaste and some other applications. OH-PCDEs are also used in industrial field such as food preservatives. Further, OH-PCDEs are formed as one of the main impurities in chlorophenols. Koistinen and co-workers identified OH-PCDEs in mussels from a river in Finland that was experimentally exposed to technical TetraCP, PCDEs and PCDD/Fs. They concluded that the TetraCP product (named Ky-5) may be the source for OH-PCDEs. Jensen et al found three OH-nonaCDE congeners in a technical PCP product. Further, 2'-OH-CDE-206, named a pre-dioxin, has been demonstrated as the precursors to OCDD (Figure 2.7).

Compared to OH-PCDEs, there is, to the best of our knowledge, no commercial source for MeO-PCDEs. The main route for the formation of MeO-PCDEs is through O-biomethylation of the corresponding OH-PCDEs. Limited data has been published on exposure of OH-/MeO-PCDEs. Available information mainly focus on abiotic samples rather than biological samples.

In this thesis, Paper V, seven OH-/MeO-PCDEs congener reference standard covering from penta- to nona-chlorinated were used to screen OH-
/MeO-PCDEs. Three nona-chlorinated OH-/MeO-PCDEs were identified. The concentration and congener profiles of these OH-/MeO-PCDEs are shown in Paper V. Again, this is the first study conducted on OH-/MeO-PCDEs exposure in wildlife in China.

**Highly chlorinated biphenyls**

PCBs with eight to ten chlorines are referred to and abbreviated as OND-PCBs in this thesis. They are not present in the major Arochlor product such as Arochlor 1242 and 1254. In contrast, Kannan et al.\(^\text{122}\) reported OND-PCBs in sediment and soil in Georgia, and they suggested that contamination could be due to the usage of Aroclor 1268 (containing 85% highly chlorinated biphenyls). In addition, CB-209, and nonaCBs has been detected in the phthalocyanine pigments.\(^\text{80,123}\) Chlorinated benzene is used as reagent medium and tetraCBs to hexa-CBs may be formed through a free radical mechanism. In the next step, nona and decaCB could be formed as a byproduct by the transformation from phthalocyanine blue to phthalocyanine green pigment by perchlorination.\(^\text{123}\) Moreover, CB-209 was also suggested as a byproduct during the purification of titanium dioxide.\(^\text{124}\) Chlorine gas and carbon are used to separate titanium from its ores. In this process, the feedstock (FeTiO\(_3\)) is chlorinated over 1000°C, forming titanium tetrachloride. The titanium tetrachloride is purified by distillation, followed by subsequently oxidized in an oxygen flame or plasma to form pure titanium dioxide as white pigment.

OND-PCBs are rarely reported in the environmental media. Nevertheless, high contribution of CB-209 (44% of total PCBs) was reported in freshwater fish from northeast Liaohe river, China.\(^\text{125}\) OND-PCBs were also reported to be present in aquatic species from e-waste recycling site in South China.\(^\text{126}\)

In this thesis, the pattern of OND-PCBs are reported in Chinese pond herons (Paper III) and further quantified in paddy filed species (Paper III) and in bird eggs (Paper IV).

**Dicofol**

Dicofol (trade name: Kelthane) is an organochlorine acaricide, which has been used to protect citrus and cotton cultivations.\(^\text{127,128}\) The estimated global usage of Dicofol from 2000 to 2012 was 28 000 tonnes, and 77% of the total was consumed in Asia.\(^\text{42}\) China is the largest producer and consumer of dicofol, with 69% of the estimated global production amount
during this period. Dicofol is synthesized from technical DDT (Figure 2.8). Qiu et al. mentioned that the high residue of DDTs in Taihu lake environment in China may be due to the production of dicofol from technical DDT mixture. They also suggested that high 2,4′-DDT/4,4′-DDT ratio could be used as an indicator to assess the contamination source of DDTs.

Dicofol has been proposed as a new POPs in the SC. However, the main argument on whether to restrict and/or prohibit dicofol is lack of evidence that dicofol is persistent. It has been demonstrated that dicofol is decomposed to DCBP in alkaline and/or thermal condition. These reaction lead to the reporting data on exposure levels of dicofol in environmental media were very limited.

![Figure 2.8. Pathway for synthesis of 4,4′-dicofol from 4,4′-DDT](image)

In this thesis a new method for analyses of dicofol and DCBP is presented (Paper II).

### 2.4 Wildlife assessed

A dozen wildlife species have been included in the studies in Paper I-VI. A clip art picture of them is presented on the cover page of the thesis. The description of each species, e.g. living condition, diet, geographic distribution, is briefly presented in Table 2.3.
Table 2.3. Description of wildlife species addressed in the thesis. The information is primarily from Wikipedia (https://en.wikipedia.org)

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic distribution</th>
<th>Diet</th>
<th>Living condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blue mussel</strong> (<em>Mytilus edulis</em>)</td>
<td>Worldwide</td>
<td>Phytoplankton and other microscopic sea organisms</td>
<td>Attached to hard surface</td>
</tr>
<tr>
<td><strong>Clams</strong> (<em>Cyclina sinensis, Ruditapes philippinarum, Sinomovacula constricta</em>)</td>
<td>Coastal area of eastern Asia</td>
<td>Plankton</td>
<td>Buried in mud or sand</td>
</tr>
<tr>
<td><strong>Snail</strong> (<em>Bellamya aeruginosa</em>)</td>
<td>Freshwater ecosystems in China</td>
<td>Detritus and humus</td>
<td>On surface sediments</td>
</tr>
<tr>
<td><strong>Asian swamp eel</strong> (<em>Monopterus albus</em>)</td>
<td>Mainly in Asia</td>
<td>Shrimp, crayfish, worms, insects and occasionally detritus</td>
<td>In freshwater environment, e.g. ditches, ponds, streams and rice paddies</td>
</tr>
<tr>
<td><strong>Dark-spotted frog</strong> (<em>Pelophylax nigromaculatus</em>)</td>
<td>East Asia</td>
<td>Insects and worms</td>
<td>Live in or near stagnant or slow-moving water</td>
</tr>
<tr>
<td><strong>Asiatic toad</strong> (<em>Bufo gargarizans</em>)</td>
<td>East Asia</td>
<td>Earthworms, snails and insects</td>
<td>Prefers humid areas, but live in most habitats incl. grasslands, open forests, cultivated areas</td>
</tr>
<tr>
<td><strong>Short-tailed mamushi snake</strong> (<em>Gloydius brevicaudus</em>)</td>
<td>China and the Korean Peninsula</td>
<td>Fresh-water fish, frogs, lizards, birds and rodents</td>
<td>Plain and hilly areas and mainly habitat in the grass mound</td>
</tr>
<tr>
<td><strong>Chinese pond heron</strong> (<em>Ardeola bacchus</em>)</td>
<td>East Asia</td>
<td>Insects, fish and crustaceans</td>
<td>In shallow fresh and salt water wetlands and ponds</td>
</tr>
<tr>
<td><strong>Black-crowned night heron</strong> (<em>Nycticorax nycticorax</em>)</td>
<td>Throughout a large part of the world</td>
<td>Small fish, crustaceans, frogs, aquatic insects, and small mammals</td>
<td>During the day they rest in trees or bushes; in the evening stands at the water edge for prey</td>
</tr>
<tr>
<td><strong>Peregrine falcon</strong> (<em>Falco peregrinus</em>)</td>
<td>Worldwide</td>
<td>Medium-size birds, small mammals, small reptiles and inserts</td>
<td>Nearly everywhere on Earth, except polar regions</td>
</tr>
<tr>
<td><strong>Whiskered tern</strong> (<em>Chlidonias hybrid</em>)</td>
<td>South Europe, North Africa, Asia and Australia</td>
<td>Small fish, insects, crustaceans, and amphibians</td>
<td>Inhabit in lakes, reservoirs, estuaries, and coastal marshes</td>
</tr>
</tbody>
</table>
3. Methodology

3.1 Samples and sampling information

Sample selection and collection is the first step in the experimental work for assessment of organic contaminants exposure. Selection of biological species is critical for environmental monitoring of pollutants. Low trophic level organisms are typically representative of specific areas of concern and more reflective of local environment, and thus they are often selected to reveal temporal and spatial trends of pollutants from local sources and specific remediation actions. High trophic level organisms tend to accumulate high level of persistent pollutants through their food webs and have a great potential for screening of emerging environmental contaminants. In addition, these high trophic level organisms are commonly selected for retrospective studies. A number of biological factors, e.g. lipids, age, size and sex, will have influence on concentrations of contaminants in wildlife and humans. Lipid would be of importance to those contaminants that partition preferentially into the lipid portion of biota e.g. DDT and PCBs, but would not for those that are rather hydrophilic or rapidly metabolized, e.g. PFOS, esters, and PCP. The age of the organism is commonly covariate with the size (e.g. weight and length). Sex is to consider, such as for top predators due to characteristics specific for the male and female.

The selection of tissue type is important when designing experiments and monitoring studies. For lower trophic level organisms (e.g. mussel and snail), in most cases the whole soft tissue is the only practical option for analysis. For higher trophic level organisms (e.g. predator fishes, birds and mammals), the selection of tissue depends on target compounds and the objectives of the monitoring program. In general, blood samples are used for analysis of phenolic compounds whereas the whole tissue or specific organ (e.g. liver) for neutral compounds. Specifically, for oviparous organisms (e.g. bird), eggs have been widely applied for chemical analysis. This is because they are readily accessible and the contaminants level reflects the level of the maternal bird.
Analysis of biological samples as a pool or individually is a matter of discussion and planning to meet the requirement of each study. Pooling of samples is necessary if the individuals do not provide sufficient amount of material. A motive for pooling is to reduce the cost as the cost for chemical analysis is commonly higher than the costs of collecting samples. However, individual samples with extreme value could not be found by pooling samples. This is a critical issue once the monitoring program aims for risk assessments. In addition, the potential co-variates relationship could not be readily assessed (e.g. age, size and trophic level). Moreover, from retrospective study point of view, it is preferable to store individual samples.

When designing a long-term environmental monitoring program, it is important to take both Type I and Type II error into account. Type I error ($\alpha$) is defined as the risk to falsely reject a true null hypothesis, whereas Type II error ($\beta$) is defined as the false not to reject a false null hypothesis. Statistical power (1-$\beta$) is the probability to detect a trend or a difference when it actually occurs. More details on statistical power are introduced in Chapter 3.5. It is noteworthy that most scientists prioritize the concern of Type I error but do not seem to be as concerned about Type II error, but still may result in unjustified conclusions.

In short, it is obvious that there are many factors to consider prior to selection of biological samples for exposure assessment for further development of monitoring programs. Hence there is no standardized strategy for setting up such programs, influenced by differences among organisms, target compounds, and objective of the programs. The data presented in Table 3.1 summarize samples and sampling information for matrices analyzed and presented in Paper I-VI. Different trophic levels of wildlife, from invertebrates (i.e. mussel and clam) to top predators (falcon) of the food webs are presented in these papers c.f. Table 3.1. Different tissues (i.e. whole tissue, muscle and eggs) were used for chemical analysis. Additionally, the sampling locations cover typical eco-system in YRD. Samples in Paper I are from selected ocean sites; samples in Paper III are from paddy field and in the vicinity of a chemical industry park southeast of Shanghai; and sample in Paper IV-VI are from fresh water lakes. This serves for the individual aim of each study but together contributes to establish a sustainable environmental monitoring program in the YRD.
Table 3.1. Description of sampling and samples in relation to each of the Papers, I-VI.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sampling time</th>
<th>Location</th>
<th>Sample &amp; number</th>
<th>Tissue</th>
<th>Analytes^a (Paper#)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue mussels (<em>Mytilus edulis</em>)</td>
<td>July 2010,</td>
<td>North Yellow Sea,</td>
<td>Pool (&gt;20)^b</td>
<td>Whole tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td>July 2011</td>
<td>East China Sea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clam (<em>Cyclina sinensis</em>)</td>
<td>July, 2011</td>
<td>South Yellow Sea</td>
<td>Pool (&gt;30)^b</td>
<td>Whole tissue</td>
<td>OCPs, BFRs (Paper I)</td>
</tr>
<tr>
<td>Clam (<em>Ruditapes philippinarum</em>)</td>
<td>July, 2011</td>
<td>South Yellow Sea</td>
<td>Pool (&gt;30)^b</td>
<td>Whole tissue</td>
<td></td>
</tr>
<tr>
<td>Clam (<em>Sinonovacula constricta</em>)</td>
<td>July, 2011</td>
<td>South Yellow Sea</td>
<td>Pool (&gt;30)^b</td>
<td>Whole tissue</td>
<td></td>
</tr>
<tr>
<td>Asiatic toad (<em>Bufo gargarizans</em>)</td>
<td>July, 2011</td>
<td>Shanghai</td>
<td>Pool (8)</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>Chinese pond heron (<em>Ardeola bacchus</em>)</td>
<td>July, 2011</td>
<td>Shanghai</td>
<td>3</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>Peregrine falcon (<em>Falco peregrinus</em>)</td>
<td>July, 2011</td>
<td>Shanghai</td>
<td>3</td>
<td>Muscle</td>
<td>OHCs^c (Paper III)</td>
</tr>
<tr>
<td>Asian field eel (<em>Monopterus albus</em>)</td>
<td>July, 2011</td>
<td>Shanghai</td>
<td>Pool (5)</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>Short-tailed mamushi snake (<em>Gloydius brevicaudus</em>)</td>
<td>July, 2011</td>
<td>Shanghai</td>
<td>3</td>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>Black-crowned night heron (<em>Nycticorax nycticorax</em>)</td>
<td>April, 2014</td>
<td>Tianmu lake</td>
<td>10</td>
<td>Egg</td>
<td>OCPs, BFRs (Paper IV); PCP, PCDD/Fs, PCDE, OH-/MeO-PCDEs (Paper V)</td>
</tr>
<tr>
<td>Whiskered tern (<em>Chlidonias hybrida</em>)</td>
<td>May, 2014</td>
<td>East Taihu lake</td>
<td>10</td>
<td>Egg</td>
<td></td>
</tr>
<tr>
<td>Snail (<em>Bellamya aeruginosa</em>)</td>
<td>April, 2014</td>
<td>Tianmu lake</td>
<td>44</td>
<td>Whole tissue</td>
<td>PCBs and PBDEs (Paper VI)</td>
</tr>
</tbody>
</table>

^a. The full name of analytes given by abbreviations are: OCPs: organochlorine pesticides; BFR: brominated flame retardants; OHCs: organohalog en compounds; PCP: pentachlorophenol; PCDD/Fs: polychlorinated dibenzo-p-dioxins/polychlorinated dibenzo furans; OH-/MeO-PCDEs: hydroxylated polychlorinated diphenyl ethers/methoxylated polychlorinated diphenyl ethers; PCBs: polychlorinated biphenyls; and PBDEs: polybrominated diphenyl ethers.

^b. Three samples from the same pool were analyzed in parallel.

^c. More than 100 OHCs were screened in Paper III but only a few groups were selected for quantification. Please see Paper III for the full documentation regarding the analytes.
### 3.2 Sample pretreatment

After sampling, all samples were carefully stored until chemical analysis was initiated. A summary of analytical procedures including extraction, clean-up, separation and instrumental analysis are presented in Table 3.2.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraction</td>
<td>LLE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>LLE</td>
<td>LLE</td>
<td>LLE</td>
<td>LLE</td>
<td>LLE</td>
</tr>
<tr>
<td>Clean-up</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>GPC</td>
<td>GPC</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Separation</td>
<td>No</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>KOH&lt;sup&gt;c&lt;/sup&gt;</td>
<td>KOH</td>
<td>KOH</td>
<td>No</td>
</tr>
<tr>
<td>Column clean-up</td>
<td>SiO&lt;sub&gt;2&lt;/sub&gt;:H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>SiO&lt;sub&gt;2&lt;/sub&gt;:H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>No</td>
<td>No</td>
<td>SiO&lt;sub&gt;2&lt;/sub&gt;:H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Fractionation</td>
<td>No</td>
<td>No</td>
<td>deactivated SiO&lt;sub&gt;2&lt;/sub&gt;:H&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Instrument</td>
<td>GC-ECD, GC-LRMS&lt;sup&gt;(ECNI)&lt;/sup&gt;</td>
<td>GC-ECD, GC-LRMS&lt;sup&gt;(ECNI)&lt;/sup&gt;</td>
<td>GC-ECD, GC-LRMS&lt;sup&gt;(ECNI)&lt;/sup&gt;</td>
<td>GC-LRMS (ECNI/EI)</td>
<td>GC-LRMS (ECNI)</td>
<td>GC-LRMS (EI)</td>
</tr>
</tbody>
</table>

<sup>a</sup> liquid-liquid extraction; <sup>b</sup> concentrated sulfuric acid treatment; <sup>c</sup> potassium hydroxide partitioning.

Notice: Further information on PCDD/Fs analysis are presented in Paper V.

### 3.2.1 Extraction and lipid determination

The OHCs included in the thesis are either lipophilic or proteinophilic. There are numerous extraction methods that have been developed and used for retrieving the analytes, as e.g. reviewed by Xu and coauthors. One of the most commonly used methods is liquid-liquid extraction as described by Bligh-Dyer and coworkers using a mixture of chloroform and methanol to extract lipids from fish. Jensen et al developed an extraction method by using n-hexane and acetone, and the idea behind this was to avoid halogenated solvent in the clean-up process. However, this initial Jensen method is not effective enough to extract chemicals from tissues with low lipid content (e.g. cod muscles). To solve this problem, Jensen et al substituted acetone with 2-propanol and partly n-hexane with diethyl ether, allowing a better extraction efficiency of phospholipids. Later, this method was even further improved also for extraction of phenolic
analytes. This was achieved by changing the ratio of n-hexane and diethyl ether from 9:1 to 3:1. The most recent Jensen method was applied to all of the studies in Paper I-VI with minor modification. All lipid weight determinations were done gravimetrically.

3.2.2 Lipid removal

All lipids in an extract, e.g. triglycerides, cholesterol, glycolipids and phospholipids, interfere in the chromatographic analysis, with the analytes, due to similar chemical properties. Both non-destructive and destructive clean-up methods have been used in this thesis for lipid removal.

The destructive method is aimed to enable the removal of the lipids but still not the analytes. There are two destructive methods used for lipid removal in the thesis, e.g. either use of concentrated sulfuric acid or by application of acidic silica gel columns. By using sulfuric acid, the target compounds, e.g. PCBs, PBDEs and DDTs are unaffected whereas lipids are destroyed due to the strong oxidizing property of sulfuric acid. Further, sulfuric acid acts as a Lewis acid to form a complex with a Lewis base, e.g. sulfone-containing compounds. In Paper I, III and VI, the samples were cleaned up on a silica gel column impregnated with sulfuric acid (2:1, w/w) to get rid of any remaining lipids.

Non-destructive methods are aimed to separate lipids from target compounds by making use of distribution coefficient and/or chromatographic properties. Gel permeation chromatography (GPC) was used in Papers IV and V to remove lipids due to the high lipid content in the bird eggs. Separation of lipids and contaminants by using GPC is based on molecular size of the chemicals in an extract. Large molecules (e.g. lipids) are quickly excluded from the stationary phase, which consists of a porous styrene divinylbenzene (with 3% cross linkage) phase. Small molecules diffuse into the pores of the beads and thus take a longer time for passing through the GPC column.

3.2.3 Separation of substances groups

*Separation of phenolic and neutral compounds*

Several compounds (e.g. PCP, OH-PCDEs), considered as endocrine disrupters, contain a phenolic group, i.e. a slightly acidic functional group. The traditional Bligh-Dyer extraction method shows a poor extraction efficiency of phenolic compounds as reported by Jensen et al. Hovander
and co-workers developed a method for separation of halogenated phenolic compounds from neutral compounds in blood plasma. The separation was achieved by potassium hydroxide partitioning (0.5 M potassium hydroxide in 50 % ethanol). The phenolic compounds are re-extracted to an organic solvent by adding hydrochloric acid (2 M). It is noteworthy that this procedure need to proceed without interruption to avoid degradation of the neutral compounds labile to alkali.

The chromatographic behavior of phenolic compounds in gas chromatographic systems may not be satisfactory due to the interactions between phenolic compounds and the stationary phase. An option for improved chromatography is to derivatize the phenols. Hence diazomethane was synthesized in house and used to form methylations. Methoxylated compounds are resistant to sulfuric acid treatment and suitable for GC analysis. The mechanism of diazomethane methylation is visualized in Figure 3.1. CATION: Diazomethane is carcinogenic and explosive. Therefore, handling should be done with great care. The use of diazomethane within the frame of this thesis was approved by the Swedish work environment authority and in accordance with AFS 2005:17 (No IMS 2009/40104).

Another option is to use liquid chromatography coupled to mass spectrometer (LC/MS). The drawback is probably the higher limit of quantification (LOQ) in LC and separation in LC is not as good as obtained by using GC.
Separation of dicofol and dichlorobenzophenone

Separation of DCBP from dicofol and other neutral compounds was achieved by sulfuric acid treatment (Paper II). The mechanism is based on the fact that DCBP is a Lewis base forming complex with concentrated sulfuric acid (Lewis acid). By diluting the concentrated sulfuric acid, the DCBP can be re-extracted to an organic solvent as described for analysis of aryl methyl sulfones.\textsuperscript{143} Accordingly, DCBP can be retrieved for further chemical analysis. In the previous studies, dimethyl sulfoxide (DMSO) was used to separate methylsulfonyl compounds from neutral compounds.\textsuperscript{146} This method was tested (Paper II) but the result was not satisfactory, i.e. both dicofol and DCBP partitioning into the DMSO phase. Accordingly DMSO partitioning was not suitable for meeting the aims of the study (Paper II).

Separation of chlorinated paraffins from other OHCs

As mentioned previously, CPs consists of extremely complex mixtures of conformational isomers, diastereomers in series of alkanes with various chain lengths (c.f. Chapter 2). Hence, CPs covers a large area in GC chromatograms and co-elutes with many other OHCs. Therefore, it is in principle necessary to separate them from other analytes.

In this thesis, a silica gel column deactivated with 5% water was used for this purpose.\textsuperscript{147} In Paper III, deactivated silica gel (3% water) was applied to separate CPs from highly nonpolar compounds. The first fraction was eluted with n-hexane and contains PCBs, Mirex and HCB. CPs were recovered in the second fraction eluted with n-hexane:diethyl ether (3:1 v/v). However, slightly polar compounds, such as HCHs, chlordane, PBDEs and toxaphene elute in the same fraction as the CPs. HCHs elutes early as shown in the GC chromatograms and before the CPs starts to elute and accordingly not creating any problems with the HCHs. PBDEs are analyzed in this thesis by GC-MS detecting the bromide ions (m/z 79 and 81), which do not interfere with assessing the CPs. Toxaphenes were not detected in the samples. Chlordanes were only detected at very low concentrations and accordingly not creating any problem in the analysis of CPs.

Alternative methods for fractionation of CPs e.g. by using Florisil (1.2% water) have been described.\textsuperscript{148} Recently, a few zeolite products (e.g. zeolite 4Å, zeolite 13X and ZSM5) were tested for separations of SCCP and other OHCs.\textsuperscript{149} Interestingly, zeolite 13X resulted in total immobility of the SCCPs on the zeolite products whereas the OHCs were eluted out of the
column. However, it was not possible to elute the CPs with any of the solvents tested so far.\textsuperscript{149} In another effort, Fridé n and coworkers developed a photolytic clean-up method for CP analysis.\textsuperscript{150} Halogenated aromatic compounds such as PCBs, DDTs were destroyed immediately. However, pesticides such as toxaphene and chlordanes were only partly degraded even after long exposure time.\textsuperscript{150}

3.3 Instrumental analysis

Analyses of OHCs in this thesis were performed by GC-ECD and/or GC-MS using different types of injectors. More details on each of the techniques are briefly discussed below.

\textit{GC-ECD (split/splitless)}

Electron capture detector (ECD) has been widely used for analysis of OHCs in environmental samples ever since the 1960’s because of its high sensitivity. GC-ECD is used in this thesis for quantitative analysis of OCPs and PCBs, with the exception of the OND-PCBs (Papers I, III and IV). In Paper III, OCPs and PCBs were identified by GC-LRMS, full scan prior to ECD analysis. Quantification was achieved by GC-ECD after using external calibration curves. In Paper I, a single-point calibration method was used for quantification. Nevertheless, the linearity of the ECD was checked.

In Paper III, GC-ECD was applied to assess the CPs concentration in the wildlife samples. It is noteworthy that nowadays GC-LRMS (electron capture negative ionization, ECNI) is the most commonly used technique for CPs analysis.\textsuperscript{96} However, this technique also suffers from a few drawbacks. Firstly, the instrumental response increases with the number of chlorine. In particular, compounds with chlorine number lower than five have a poor response, and cannot be reported among the CP congeners. Secondly, there may be interference between SCCPs and MCCPs when analyzed by GC-LRMS (ECNI).\textsuperscript{151} Using high resolution mass spectrometry (HRMS), with a resolution over 12 000 will however circumvent this problem.\textsuperscript{105} Recently, LC-MS method (APCI-qTOF-HRMS) was developed for CPs analysis.\textsuperscript{152} This method is particularly sensitive for MCCPs and LCCPs, indicating it could be utilized as a complementary technique for CPs quantification.\textsuperscript{152}

As Paper III is a screening study covering a number of OHCs, it was decided to use GC-ECD as a method for semi-quantification of CPs. A
number of technical CP standards from European market were assessed by GC-MS (ECNI, full scan) together with the samples. The standard that fit best was selected for quantification with GC-ECD.  

**GC-LRMS (ECNI)**

GC, coupled to a quadrupole, low resolution instrument has commonly been used for analysis of OHCs. Two ionization technique, electron impact ionization (EI) and ECNI are commonly used for OHCs. ECNI is a sensitive technique for electronegative compounds containing e.g. bromine and chlorine. In generally ECNI is a soft ionization technique where electrons emitted from the filament are retarded by a buffer gas (e.g. methane, ammonium) and form low energy electrons (thermal electrons). In general brominated compounds (e.g. PBDEs) primarily undergo dissociative ionization and form bromide anions (Br\(^{-}\), m/z: 79, 81) whereas chlorine compounds (e.g. PCBs) undergo both non-dissociative and dissociative ionization and thus form molecular ions (M\(^{+}\)), fragment ions (M-2Cl)\(^{-}\) and M\(^{+}\) ions.

In paper I, III, IV and VI, all of the bromine compounds were quantified by GC-LRMS (ECNI) using selected ion monitoring (SIM) of the bromide ions (m/z: 79, 81). In paper III and IV, OND-PCBs were quantified using the GC-MS (ECNI, SIM) monitoring two of the most abundant ions in the molecular ions (Cl\(^{-}\)) isotopic cluster.

**GC-LRMS (EI)**

EI is a robust, reproducible technique in mass spectrometry with extensive application areas. Compared to ECNI, EI is a hard ionization technique and thus may produce more fragments and less intense molecular ions. On the other hand, if the electron energy is set to 70 eV in EI the results from the analysis allow comparisons with spectra in the NIST libraries.

In Paper III, the samples were also analyzed by GC-LRMS (EI) full scan to obtain more information of fragmentation pattern for unidentified contaminants. In Paper V, GC-LRMS (EI) was applied for quantification of PCDE and MeO-PCDEs by SIM. The reasons for using EI rather than ECNI are that the fragment ions in ECNI were dominated by m/z=265 for all of MeO-nonaCDE congeners and CDE-209 while they did not give any observable molecular ions. To be able to separate 3’-MeO-CDE-207 and 4’-MeO-CDE-208 (co-eluting congeners), differences in EI fragmentation pattern was used.
**GC-HRMS (EI)**

The limit of detection using GC-HRMS is normally two to three orders of magnitude lower than that with GC-LRMS.\(^ {138} \) Consequently, GC-HRMS has been widely applied for PCDD/Fs analysis. This is primarily because PCDD/Fs concentrations are much lower than the levels of other POPs. It is also because a higher selectivity is needed due to that PCDD/Fs can interfere with other chlorinated aromatic compounds in samples. In Paper V, PCDD/Fs were analyzed by GC-HRMS (EI) with a resolution of 10 000. A 60 m column was also used to achieve better separation.

**On-column injection for GC-ECD analysis**

In Paper II, on-column injection was used for dicofol analysis because dicofol is thermally degraded in a hot split-splitless injector. It is indeed favorable to do the injections on an on-column injector as the temperature is below the boiling point of the solvent. This makes a significant difference compared to splitless injection (Paper II).

Chromatograms of dicofol, after on-column injection (A) and a normal splitless injection (B) are shown in Figure 3.2. It is clearly shown that dicofol is mainly decomposed to DCBP together with other unknown byproducts when undergoes a splitless injection. However, no DCBP was formed during on-column injection.

In Paper II, an analytical method, including liquid liquid extraction, lipid removal and separation by functional group by use of sulfuric acid and on-column injection, was applied for dicofol and DCBP analyses. The method was validated by spiking dicofol at two concentrations to cod samples. The recovery of dicofol was 65% at low dose (1.0 ng) and 77% at the higher dose (10 ng), showing a satisfactory result even if the recovery was slightly lower than for CB-200. It is noteworthy that the method is promising for dicofol analysis together with other OCPs. The on-column technique is promising for other semi-volatile but thermally unstable compounds, as well.
Figure 3.2. Chromatogram of dicofol standard by different injection techniques, A) on-column injection and B) splitless injection (degradation occurs and forms DCBP as a major decomposition product).

3.4 Quality assurance and quality control

Some of the aspects to assure and control the quality of the results are discussed below.

Surrogate Standard

Surrogate standards are commonly used for quantification. The surrogate standard needs to fulfill requirements such as: (1) structural similarity to the analytes; (2) not being present in the samples; (3) not to co-elute with any known analyte. In many cases it is difficult to find a surrogate standard fulfilling all of the requirements. As a consequence, compromises are necessary. In principle, isotopic labeled standards, preferably $^{13}$C, are an ideal choice for MS analysis. However, not all chemicals are available with stable isotope labeling and accordingly non-labeled compounds have to be used.

In Paper V, $^{13}$C-labelled PCDD/Fs were used as surrogate standards for dioxin analysis. For the other studies, BDE-138, BDE-139, CB-189, CB-200 and Dec 603 have primarily been used as surrogate standards.
Solvent blank

Solvent blank is always accompanied with the whole analytical procedure, including extraction, clean-up, and instrumental analysis, to access the contamination during the whole procedure. Some of the target analytes were detected in the blanks in Paper I-VI. For those compounds detected at low levels in the samples (e.g. PBDEs), solvent blank concentrations were subtracted from the analyte levels in the samples. If the analyte concentrations were high in the samples and low in the blanks, no adjustments of the levels were made.

LOD and LOQ

There may be different ways to define the limit of detection (LOD) and limit of quantification (LOQ). In this thesis, all of LOD and LOQ are reported as method LOD and LOQ, which consider both instrumental condition and matrix effect. LOD is defined as three times signal to noise ratio in the chromatogram. LOQ is defined as ten times signal to noise if there is no contamination in the solvent blank. Alternatively, LOQ is defined as three times the amount in the solvent blank if it presents in the solvent blank.

LOD and LOQ are of importance to statistical analysis. Considering level below LOQ as zero could results in underestimation of the results. On the contrary, considering level below LOQ as LOQ would lead to overestimation of the results. To comprise these two extreme situation, in paper V and VI, these values lower than LOD were considered as zero whereas the values higher than LOD but lower than LOQ were replaced by a certain value, i.e. half LOQ in Paper V and root square of 0.5 LOQ in Paper VI (concentration based on lipid weight).

3.5 Statistical analysis

Principal component analysis

Principal component analysis (PCA) is one of a classic multivariate method. PCA uses an orthogonal transformation to convert several types of variables (e.g. measurement or observation) into a new set of linearly uncorrelated variables. This technical can identify which characteristics vary most between individuals. The core of PCA is dimensionality reduction.

In Paper IV and V, PCA was applied to compare PCBs and PCDD/Fs
congener profiles in bird eggs with technical products and samples with identified sources, for investigating potential sources. The original data was centralized and scaled to remove the influence from the different units and gives different variables equal weighting.

Statistical power

Statistical power is defined as $1 - \beta$, where $\beta$ is the probability of making a Type II error (the risk to fail to reject a false null hypothesis). Therefore, the statistical power reflects the probability of correctly rejecting the null hypothesis (c.f. Figure 3.3).

| Table 3.3. Four possible outcomes for a statistical test of a null hypothesis.\textsuperscript{136} |
|---|---|---|---|
| Null Hypothesis ($H_0$) | True | False |
| Judgement of Null Hypothesis ($H_0$) | Not accept | Type I error ($\alpha$) | Correct ($1-\beta$) |
| | Accept | Correct | Type II error ($\beta$) |

Statistical power analysis usually contains five pieces of information. (1) $\beta$ value, or statistical power ($1-\beta$), (2) $\alpha$ value, the probability of making Type I error; (3) variability of the measurements, represented by i.e. standard deviation ($\sigma$); (4) effect size, the degree to which the observed phenomenon or response differed from the value mentioned in null hypothesis, represented by $\delta$; and (5) number of the samples or measurements ($n$). A limited sample size leads to unreliable results while the excess sample number leads to waste of resources. It should be pointed out that these five pieces of information are functionally related.\textsuperscript{153} With respect to long-term monitoring program, power analysis is commonly applied to determine the sample size necessary to generate acceptably high power (over 0.75).\textsuperscript{135, 136} In paper VI, power analysis is used to estimate sample sizes required for chemical analysis under several sampling strategies assuming $\alpha=0.05$, $1-\beta=0.8$ and 0.9 and an increasing trend of 5% a year lasting for 10 years.
Figure 3.3. Illustration of statistical power (1-\(\beta\)). The normal distribution above shows an assumed sampling distribution for a case in which the null hypothesis, \(H_0\), is true, where \(\mu_0\) is the mean value, \(z_\alpha\) is the limit for the probability of making a Type I error (\(\alpha\)). Below is an assumed observed sampling distribution for the case in which the \(H_0\) is false, where \(\mu_1\) is the observed mean value. Observed mean values in the area to the right of the line will lead to a correct rejection of \(H_0\) while mean values in the area to the left will falsely lead to accepting \(H_0\). The probability is 1-\(\beta\) and \(\beta\), respectively.

Correlation test

Correlation is used to study the relationship between two variables e.g. CB-153 vs. \(\sum_{PCBs}\) or age vs. weight. Pearson correlation test is one of the most used test to examine the correlation of two variables. It assumes that the data are normally distributed. However, in reality, the environmental contaminant data often display a right skewed distribution.\(^{135}\) There are two options when the data are not normally distributed. The first one is transforming the data in which each of the measured or observed values is e.g. log transformed or square root transformed to produce a new set of data.\(^{154}\) The other option is to use non-parametric test. In Paper IV and V, Kendall tau and Spearman correlation test were used to investigate the correlation among contaminants, respectively. The significant level was set at 5% (\(\alpha=0.05\)). These non-parametric tests commonly rank the data first,
and then examine the monotonic association between the two variables rather than the original values. As a consequence, they may be less powerful when data are normally distributed but more powerful when data contain extreme values.¹⁵⁴
4. Results and discussion

Since the results from the studies presented in Paper I-VI have been discussed in detail in each of the papers, this chapter is highlighting some major results presented in these individual articles and manuscripts. The highlights are presented in order of perceived importance under environmental exposure, below. The chapter continues with discussion on sources of the pollutants and environmental monitoring considerations.

4.1 Environmental exposure

4.1.1 Chlorinated paraffins

In Paper III, CPs were detected in a number of wildlife species from a paddy field in Jinshan district, Shanghai, contributing to over 90% of the OHCs in those terrestrial species. The highest concentrations of CPs were found in snakes, ranging from 200 000 to 340 000 ng g⁻¹ lw, followed by toad (97 000 ng g⁻¹ lw) and falcon (8 000-130 000 ng g⁻¹ lw). These concentrations are among the highest concentrations ever reported. Ma and coworkers detected comparable levels of SCCPs in invertebrates (4 800-54 000 ng g⁻¹ lw) and fish (9 700-33 000 ng g⁻¹ lw) in Liaodong Bay, North China. Even though the study area in Paper III is close to a chemical industry park, and therefore might not be representative for the whole area of the YRD, it still underscores the urgency and importance for studies of CPs (environmental exposure and ecotoxicology) in the YRD and elsewhere in China.

The results presented in Paper III should be set in perspective to significant increases of CP production, i.e. from 150 000 tonnes in 2003 to 1 000 000 tonnes in 2013. The increase of production was also indicated by limited research projects studying temporal trends of CPs in the Chinese environment. Further, a significant increase trend of SCCPs and MCCPs in marine mammals was recently reported by Zeng et al over the past decade in the South China Sea. They also found an apparent temporal shift in trend from SCCPs to MCCPs. Harada and co-workers compared SCCPs level in food samples taken from China (Beijing), Japan (Kyoto,
Okinawa and Hokkaido) and Korea (Seoul) in 1990s and 2007-2009, and found that the Chinese concentrations of SCCPs increased by two orders of magnitude, while there were no such changes in the CP levels in Japan and Korea.\textsuperscript{157}

In Paper III, CP quantifications were performed by comparison to the best chromatographic fit of a technical CP product, from the European market. According to GC-MS (ECNI, full scan) spectra, Cereclor S52 ($C_{14-17}$, 50-52 % Cl) showed the best fit with the retention times and the general chromatographic pattern of CPs in the samples. Therefore Cereclor S52 was used as the CP standard of choice for quantifications of CPs in the samples. However, the major CP products on the Chinese market are: CP-52, CP-42 and CP-70 which are mixtures of SCCPs, MCCPs and/or LCCPs.\textsuperscript{158} The numbers refer to the relative degree of chlorine in the CP products. Recently, the chromatographic congener profiles of CP-52 and CP-42 were measured by GC-LRMS. The results of these analyses have not been shown earlier but now presented in Figure 4.1. For CP 52, contributing to over 80% of national Chinese market, $C_{14}$ carbon chain length was the dominating group, followed by $C_{13}$ and $C_{15}$. This demonstrates that CP-52 is composed of both SCCPs and MCCPs. The dominating chlorination level shows binding of 7-9 chlorine atoms in each of the alkanes. A chain length with 17 carbons is the most abundant alkane in CP-42, but the product consists of alkanes in the range of decane up to heptadecane (Figure 4.1, left). Unfortunately no data on LCCPs is available due to the low sensitivity in GC-LRMS. However, according to the literature, CP-42 is dominated by LCCPs.\textsuperscript{159} Nonetheless, these additional results illustrated the complexity of CPs in the biological samples.

Figure 4.1. Relative abundance of homologous and congener profile of carbon and chlorine in technical CPs product in Chinese market by GC-LRMS (ECNI).\textsuperscript{160, 161}
4.1.2 Polychlorinated diphenyl ethers, hydroxylated polychlorinated diphenyl ethers and methoxylated polychlorinated diphenyl ethers

In Paper III, the quantitative GC-MS (ECNI) analyses of Chinese pond heron show a cluster of analytes having a fragment ion of m/z 265. This ion is indicative of substances with a phenyl ring substituted with oxygen and five chlorine atoms. In Paper V, corresponding peaks were found and identified as PCDEs according to authentic reference standards. In the bird eggs CDE-206 and CDE-209 are the most abundant PCDEs.

The median (with a range) concentrations of the PCDEs were 64 (11-450) and 160 (15-700) ng g⁻¹ lw in eggs of night heron and whiskered tern, respectively. The level was comparable to fishes (15-900 ng g⁻¹ lw) and seals (220-460 ng g⁻¹ lw) but much lower than birds (960-50400 ng g⁻¹ lw) in Finland.¹¹⁸ However, the PCDE congener profile is different from those in wildlife from Finland. They showed that hexaCDEs contributed mostly to their occurrence in pike (Esox lucius) whereas tetra- and pentaCDEs were the most abundant ones in Baltic birds.¹¹⁸ This is different from our results in the present study (Paper V) which is dominated by nona- and decaCDEs.

Seven congeners of OH-CDEs and their corresponding anisoles, MeO-CDEs were screened in bird eggs. Three congeners with nine chlorines were identified in the samples. The mean concentrations (range shown) of OH-nonaCDEs was 15 (ND-84) and 98 (ND-280) ng g⁻¹ lw in the night heron and whiskered tern eggs, respectively whereas MeO-nonaCDEs were detected in night heron and whiskered tern eggs with a mean concentration of 40 (ND-250) and 150 (14-330) ng g⁻¹ lw, respectively.

An interesting compound pattern difference was observed in OH-nonaCDEs and MeO-nonaCDE. 3’-OH-CDE-207 was the most abundant OH-PCDEs in both species while 2’-MeO-CDE-206 contributed with >80% of the ΣMeO-nonaCDE. This may be explained by 2’-OH-CDE-206 being transformed to OCDD, since 2’-OH-CDE-206 is a pre-dioxin which is a known precursor of OCDD.⁵⁴, ¹²⁰ The OH-nonaCDE congener profiles were different from those in soil and sediment from Australia, that were dominated by 2’-OH-CDE-206 and 4’-OH-CDE-208.¹²⁰
4.1.3 Polychlorinated dibenzo-\(p\)-dioxins and polychlorinated dibenzofurans

PCDD/Fs were analyzed in the bird eggs discussed above (Paper V). The median concentration of \(\sum \text{PCDDs}\) was 1 800 (240-18 400) and 9 800 (2 400-31 600) pg g\(^{-1}\) lw in the black-crowned night heron and whiskered tern eggs, respectively, and the \(\sum \text{PCDFs}\) concentrations 230 (130-1 400) and 350 (150-740) pg g\(^{-1}\) lw, respectively. The very high \(\sum \text{PCDD}\) concentrations in whiskered tern eggs are exceptional and among the highest concentrations ever reported in birds.\(^{112, 162-164}\)

The median TEQs of PCDD/Fs (WHO 2005) was 300 and 520 pg TEQ g\(^{-1}\) lw in the eggs from night heron and whiskered tern, respectively. The main contributor to the high TEQs is OCDD, followed by 1,2,3,4,6,7,8-HeptaCDD. These highly chlorinated PCDDs congeners still have low TEFs\(^{83, 84}\), i.e. several orders of magnitude less than TCDD. TCDD was reported as contributing the most in bird eggs from in Europe and North America.\(^{112, 162, 164, 165}\). It has to be pointed out that the heron eggs are consumed by humans in the local area. The TEQ levels detected in the present study were two orders of magnitude higher than the European Union guideline value in chicken eggs.\(^{166}\)

4.1.4 Highly chlorinated biphenyls

A novel PCBs contamination pattern with a higher relative content of OND-PCBs was detected in a number of species as reported in Paper III and IV. These OND-PCBs accounts for 6.9-20% of \(\sum \text{PCBs}\). To the best of my knowledge, they are the first studies reporting the entire set of sixteen OND-PCB congeners in wildlife from China. The mean concentration of CB-209 was 6.4 and 4.3 ng g\(^{-1}\) lw in black-crowned night heron and whiskered tern, respectively. Other major congeners of OND-PCBs were CB-203/196, CB-199 and CB-194. CB-209 has previously been detected in a number of bird species in China\(^{167, 168}\), contributing to >1% of total PCBs in the samples. In addition, CB-209 was detected as the most abundant PCB congener in fish from Liaohe river in Northeast China, accounting for as much as 44% to \(\sum \text{PCBs}\).\(^{125}\) Even though PCBs have been regulated in the SC since signed, 2001, this special pattern calls for the investigation of potential sources for the OND-PCBs (c.f. 4.2).

4.1.5 Legacy POPs

Some of the prioritized legacy POPs concentrations reported in individual
papers are summarized in Table 4.1 and further highlighted below.

**DDT**

Among the legacy POPs reported on in Paper I-VI, 4,4’-DDT and its metabolites 4,4’-DDE are commonly present at high concentrations in the wildlife assessed. The mean concentration of DDE varies from 160 to 490 ng g\(^{-1}\) lw in shellfish (Paper I) and 450 ng g\(^{-1}\) lw in black-crowned night heron and 320 ng g\(^{-1}\) lw in whiskered tern eggs (Paper IV). In Paper III, the DDTs are the predominated contaminants if CPs are excluded in the comparison. Such extensive contamination of DDTs is supported by other studies conducted on other matrices in China\(^{169, 170}\). Indeed, the DDTs levels presented in Paper I, III and IV are in moderate range compared with those studies in other parts of China\(^{171-173}\). Nevertheless, there is no doubt that DDT contamination is of major importance and to be considered in the YRD, as in all of China. It should be noted that DDT has been prohibited for agriculture use since 1983 in China, however, such high level in Chinese environment imply new input of DDTs into the environment.

**Industrial chemicals**

In contrast with high concentrations of DDTs, a range of other legacy POPs, including the HCHs PCBs, PBDEs and HCB, showed relatively low concentration in both shellfish (Paper I) and bird eggs (Paper IV). On the other hand, HBCDD concentrations are relatively high in both the shellfish and the bird eggs assessed, indicating a different pollution profile of this particular BFR. In Paper III, wildlife species collected near an industrial park was screened and analyzed for OHCs, the PCBs and PBDEs concentration in Chinese pond heron was in the range of 210-3300 ng g\(^{-1}\) lw and 29-210 ng g\(^{-1}\) lw. These levels are comparable to those detected in North China\(^{174, 175}\), but one order of magnitude lower than in birds from the e-Waste recycling sites in South China.\(^{168, 172}\)

CB-153 (2,2’,4,4’,5,5’-hexaCB) and BDE-47 (2,2’,4,4’-tetraBDE) have been detected as one of major congeners for PCBs and PBDEs, respectively in Paper I, III and IV. This is consistent with other studies conducted in a various wildlife species.\(^{176-178}\) It may be explained by the structural characteristic and molecular size. The chemical structures of CB-153 and BDE-47 make these substances more reluctant to metabolism than for compounds with no halogen-substituted adjacent meta-/para-position.\(^{179}\) The molecular size is optimal for bioaccumulation as the lower
halogenated congeners are more easily metabolized compare to higher halogenated PCBs and PBDEs.\textsuperscript{180}

BDE-209 was only reported in wildlife in Paper III, with low concentration compared to other PBDE congeners (e.g. BDE-47 and BDE-99). BDE-209 was examined in shellfish (Paper I) and bird eggs (Paper IV) but not quantifiable. On the contrary, BDE-209 was reported as the predominated PBDE congener in sediment and soil in YRD.\textsuperscript{181, 182} This is in line with our own results that BDE-209 is the major PBDE congener in sediments in Taihu and Dianshan lake (unpublished). Such congener composition difference could be due to a low bioavailability of BDE-209.\textsuperscript{183} Another explanation could be metabolism, i.e. debromiantion of BDE-209 to form lower BDE congeners in wildlife.\textsuperscript{184} It has been proven that BDE-209 is undergoing debromination to form lower PBDE congeners (e.g. BDE-202, -154, -153, -99, and -47).\textsuperscript{184, 185}

Interestingly, HBCDD was detected in somewhat higher concentrations than for PBDEs in shellfish (Paper I) and paddy field wildlife (Paper III). Concentrations of HBCDD in the Chinese pond heron (Paper III) were as high as those of most bird species from China.\textsuperscript{186, 187} However, these levels were lower than those reported for HBCDD in e.g. oysters and blue mussels from coastal areas of Japan\textsuperscript{188} and shrimp and fish from Western Scheldt Estuary, Europe\textsuperscript{189}. HBCDD is indeed a POP in the SC. The results in this thesis imply that HBCDD and PBDEs are equally important for environmental monitoring program in the YRD.

4.2 Potential sources

4.2.1 PCDD/Fs, PCDEs and OH-/MeO-PCDEs

Previous studies showed that PCDD/Fs can be produced from several different sources.\textsuperscript{13} Multivariate analysis techniques have been a common approach to promote the interpretation of PCDD/Fs sources. In Paper V, principal component analysis was used to compare PCDD/Fs congener pattern in bird eggs with those patterns found in specific sources from literatures. The PCA result showed that bird egg samples were close to sources such as atmospheric deposition and pentachlorophenol. This is in accordance with previous results from offshore and coastal surface sediment from the Baltic Sea.\textsuperscript{90, 190} Baker and Hites\textsuperscript{94} found that annually PCDD/Fs atmospheric deposition amount was much higher than its combustion sources. The authors suggested that synthesis of OCDD from
Table 4.1. Results of some prioritized OHCs in wildlife from Paper I-VI (mean and range of concentration based on lipid weight ng g$^{-1}$ lw).

<table>
<thead>
<tr>
<th></th>
<th>Blue mussel Paper I (n=6)$^a$</th>
<th>Clam Paper I (n=9)$^a$</th>
<th>Chinese pond heron Paper III (n=3)</th>
<th>Peregrine falcon Paper III (n=3)</th>
<th>Snake Paper III (n=1)$^a$</th>
<th>Toad Paper III (n=1)$^a$</th>
<th>Frog Paper III (n=1)$^a$</th>
<th>Eel Paper III (n=1)$^a$</th>
<th>Night heron Paper IV (n=10)</th>
<th>Whiskered tern Paper IV (n=10)</th>
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<tr>
<td>4,4'-DDE</td>
<td>180</td>
<td>260</td>
<td>5900 (390-17000)</td>
<td>500 (130-12000)</td>
<td>320 (210-430)</td>
<td>590</td>
<td>23</td>
<td>760</td>
<td>450 (280-650)</td>
<td>320 (240-480)</td>
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<tr>
<td>4,4'-DDTs$^b$</td>
<td>340</td>
<td>590</td>
<td>6400 (430-17000)</td>
<td>520 (150-12000)</td>
<td>390 (260-490)</td>
<td>680</td>
<td>61</td>
<td>970</td>
<td>520 (290-780)</td>
<td>360 (270-610)</td>
</tr>
<tr>
<td>β-HCH</td>
<td>2.8</td>
<td>3.7</td>
<td>32 (0.53-93)</td>
<td>20 (5.7-48)</td>
<td>140 (53-270)</td>
<td>69</td>
<td>190</td>
<td>29</td>
<td>300 (150-850)</td>
<td>79 (30-270)</td>
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<tr>
<td>Mirex</td>
<td>ND</td>
<td>1.6</td>
<td>190 (29-420)</td>
<td>44 (21-77)</td>
<td>20 (9.4-32)</td>
<td>12</td>
<td>0.35</td>
<td>3.4</td>
<td>94 (17-340)</td>
<td>41 (9.3-160)</td>
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<td>HCB</td>
<td>2.1</td>
<td>2.8</td>
<td>1.1 (0.02-32)</td>
<td>1.1 (0.08-3.0)</td>
<td>4.4 (0.90-9.0)</td>
<td>9.9</td>
<td>11</td>
<td>0.05</td>
<td>210 (94-380)</td>
<td>130 (93-190)</td>
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<td>CB-153</td>
<td>5.6</td>
<td>2.6</td>
<td>200 (31-480)</td>
<td>61 (36-83)</td>
<td>21 (7.6-36)</td>
<td>15</td>
<td>1.9</td>
<td>7.8</td>
<td>110 (39-280)</td>
<td>120 (32-330)</td>
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<tr>
<td>CB-209</td>
<td>12 (4.2-26)</td>
<td>6.2</td>
<td>4.1 (9.7)</td>
<td>2.4 (0.99-5.2)</td>
<td>2.2</td>
<td>0.64</td>
<td>0.59</td>
<td>6.4</td>
<td>3.5-16)</td>
<td>4.3 (1.3-15)</td>
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<tr>
<td>ΣPCBs$^c$</td>
<td>13</td>
<td>12</td>
<td>800 (100-1900)</td>
<td>200 (110-300)</td>
<td>56 (23-96)</td>
<td>36</td>
<td>6.9</td>
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<td>310</td>
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<td>ΣOND-PCBs$^d$</td>
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<td></td>
<td>47 (21-100)</td>
<td>38 (11-150)</td>
</tr>
<tr>
<td>BDE-47</td>
<td>1.7</td>
<td>1.7</td>
<td>1.1 (0.57-2.1)</td>
<td>2.5 (1.1-4.7)</td>
<td>0.17 (0.12-0.28)</td>
<td>0.38</td>
<td>0.22</td>
<td>1.9</td>
<td>4.7 (1.2-15)</td>
<td>45 (6.8-260)</td>
</tr>
<tr>
<td>BDE-99</td>
<td>0.97</td>
<td>0.50</td>
<td>0.93 (0.80-1.0)</td>
<td>3.4 (2.4-5.2)</td>
<td>0.35 (0.30-0.44)</td>
<td>0.44</td>
<td>0.29</td>
<td>1.3</td>
<td>3.7 (0.65-19)</td>
<td>24 (2.1-200)</td>
</tr>
<tr>
<td>ΣPBDEs$^e$</td>
<td>4.4</td>
<td>4.1</td>
<td>93 (59-210)</td>
<td>27 (22-30)</td>
<td>28 (22-36)</td>
<td>24</td>
<td>8.1</td>
<td>34</td>
<td>43 (19-150)</td>
<td>99 (15-480)</td>
</tr>
<tr>
<td>HBCDD</td>
<td>27</td>
<td>38</td>
<td>146 (39-330)</td>
<td>6.8 (3.7-12)</td>
<td>80 (1.2-230)</td>
<td>14</td>
<td>3.1</td>
<td>16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND: not detected
a. number of pool samples
b. sum of 4,4'-DDT, 4,4'-DDE and 4,4'-DDD
e. sum of total PCDE congeners. The congeners are different in different studies.
PCP in atmospheric condensed water may be the reason for this deficit\textsuperscript{94}, which seems as a logical explanation.

Spearman correlation test was conducted on PCDEs, OH-/MeO-PCDEs, which are the major impurities and byproducts from PCP products, and PCDD/Fs. A correlation (Spearman correlation test, \( p<0.05 \)) was detected between 2\('\)-MeO-CDE-206 and OCDD, OCDF, CDE-209 and 2\('\)-OH-CDE-206. 3\('\)-MeO-CDE-207 also presented a correlation with OCDD, and OCDF, implying OCDD/Fs might have the same sources as MeO-nonaCDEs. As mentioned, MeO-nonaCDEs are supposed to be transformed from OH-nonaCDEs, which can be formed via dimerization of PCP.

In conclusion, the results from multivariate analysis and correlation test indicated that the contaminants reported in Paper V are directly or indirectly related to PCP production and application in the YRD. However, some caution may apply in drawing too firm conclusions about PCP being the single source since e.g. household coal burning, agricultural straw open burning may contribute with some of the OCDD and heptaCDD.\textsuperscript{85,89} It would be valuable to be able to get access to technical products of PCP for analysis and comparison to the contaminant patterns in wildlife in general but in particular with the bird eggs assessed herein (Paper V). Paper V provides a contribution for source identification of PCDDs/Fs even if the technical product used in the analyses were not identical to the ones actually used and the results may also be biased to some extent by the birds' metabolism and transformations along the path in the food web. But OH-/MeO-PCDEs, and PCDEs can indeed be regarded as early warning substances for dioxins, in particular for OCDD as shown here.

### 4.2.2 Highly chlorinated biphenyls

As mentioned in Chapter 2, there are a few origins for OND-PCBs. Hu and Hornbuckle analyzed all of 209 PCB congeners in commercial paint pigments around Great Lakes and found that phthalocyanine green pigment contains OND-PCBs.\textsuperscript{123} This is consistent to a similar study in Japan by Anezaki and Nakano, in which CB-209 was the predominant congener in phthalocyanine green pigment product sold on Japanese market.\textsuperscript{80} China is known as the largest producer and exporter of classic organic pigment in the world with the annual production amount of 185 000 tonnes in 2008.\textsuperscript{191} However, the research on PCBs in pigment product is limited in China and elsewhere. To best of our knowledge, there is only one study on concentration and distribution of PCBs in azo-type pigment in China. In that study, CB-11 was the dominant PCB congener whereas CB-209 was detected in trace amount only.\textsuperscript{191}
CB-209 was previously reported in various bird species from northern China, and suggested to be originated from technical product similar to Aroclor 1270. In addition, Luo and coauthors detected CB-209 at levels of up to 12200 ng g\(^{-1}\) lw in White-breasted water-hen from an extensive e-waste recycling region in South China. Further, we found a significant correlation between CB-209 and higher BDE congeners in sediment from Taihu lake (Unpublished). The latter data implies that OND-PCBs may be used as flame retardants.

4.2.3 DDT and dicofol

Although DDT was banned as a pesticide, it has been continuously used in other areas. One of the main applications of DDT is as a starting material to produce dicofol. However, analysis of dicofol is a problematic issue due to its low thermal stability as discussed above and in Paper II.

Qiu et al found that the ratio between 2,4’-DDT and 4,4’-DDT in commercial dicofol product in Chinese market was around 7, which is close to the air samples in Taihu lake area, but different from technical DDT product with a ratio of 2,4’-DDT/4,4’-DDT about 1/4. The authors purposed that ratio of 2,4’-DDT/4,4’-DDT can be used as an indicator for “dicofol type DDT pollution”. However, the ratio between 2,4’-DDT and 4,4’-DDT observed in Paper I and IV were much lower than 7. Therefore, it might be suspected that apart from dicofol production, such high DDTs level could be linked to other sources such as illegal use of DDT as pesticides or anti-fouling paints.

The ratio between 4,4’-DDT and \(\sum\)DDEs has been commonly used to assess the pollution type of DDT. In Paper I and IV, this ratio was calculated and shown below 0.5, indicating a historic source. However, Qiu et al stated that \(\alpha\)-Cl 4,4’-DDT, which is the intermediate product in dicofol production process from 4,4’-DDT, can form 4,4’-DDE. Consequently, it leads to a low ratio of 4,4’-DDT/\(\sum\)DDTs and may be misleading in the evaluation of the sources of DDT contamination.

4.3 Environmental monitoring consideration

Monitoring of biological samples is aimed at monitoring environmental quality, health protection and promotion of exposure and risk assessment. The environmental monitoring programs serve as a base for management and control of actions taken, but are also important for the discovery of emerging contamination. An important remark: the variability from biological sample is commonly higher compared to variability from the chemical analytical process, requiring a considerate sampling design and strategy. Nonetheless, biological monitoring is valuable as it reflects the bioavailable part of the
contamination and how environmental contamination influences wildlife, and gives a chance for extrapolations to humans. Several consideration issues on establishment of environmental monitoring program using biological samples are discussed below.

4.3.1 Spatial and temporal trend monitoring efforts

Spatial distribution monitoring programs aim to overview regional and/or local differences of contaminants. Spatial monitoring programs can be used to detect hot-spots and potential sources. Further, this methodology is promoting contaminant assessments in relation to distance from known point sources, promoting the understanding of pollutant transport and environmental fate. In paper I, shellfish was used to assess the contamination situation in sea-water systems along a part of the Chinese coast. These species have a low mobility and can be used to reflect the local environment condition. However, different species were used, i.e. blue mussel in Weihai and Zhoushan city whereas clam in Nantong city, which may confound the spatial assessment. In a later study (unpublished), mussels and snails were collected from a few ponds in YRD for OHCs analysis. The results showed that snails had a similar contamination concentration and composition as the mussels. Since snails are more readily available in the YRD fresh waters, it is possible to replace mussels with snails in our monitoring program (c.f. Paper VI).

It is questionable to use species at high trophic level for spatial trend studies, as many factors, including mobility, migration, availability of food sources and food web that can distort the observations. Another issue that needs to be emphasized is how representative the sampling is for assessing spatial distribution from one single occasion (year) and the analyses of these samples? It is difficult to know if sample from this certain year is in the range of a “control chart” or being an “outlier”. Therefore, several years of analyses from each sampling site may be preferable to obtain representative information even though it is costly and time-consuming. Comparisons and interpretations of the results need to be performed with caution.

Temporal trend monitoring allows detection and calculations of trends of contaminant effects or concentrations over time. Compared to spatial trend analysis, it is easier to select species for temporal trend analysis as variation on such species (e.g. migration, living habits) can be offset. Each sample should be independent and representative whereas sample size should be large enough to reach a certain statistical power (c.f. Paper VI). However, it has to be compromised with the study cost. It should be pointed out that with-in year sample size of top predators is usually low due to the small populations.
The experience from the Swedish monitoring programs show significant contaminant decreases after restriction and prohibition, to be interpreted as environmental quality improvements. It may open up for changes in the monitoring efforts and priorities to gain new knowledge and possibly reduce the program costs. Since a large number of chemicals are being produced and used in numerous different applications it is important to see what may be introduced into the temporal trend studies of the future. This is of importance for the Chinese situation as well. Numerous studies have dealt with environmental exposure to OHCs over the past decades in China. However, only limited studies have considered temporary trends. To improve this situation, well-designed monitoring programs, including specimen bank collection and storage of the samples is required.

4.3.2 Wildlife exposure vs. Human risk

Environmental monitoring programs include abiotic and biological matrices, the latter including a several different species of wildlife (e.g. guillemot eggs, herring mussel, lake trout) and can be divided into two categories in general. The first category is aimed to monitor environmental contamination and exposure level to wildlife while the other category is specifically aimed at human monitoring and protection. Because of the objective difference, the study design between those two categories is to some extent different. As illustrated in Chapter 3.1, there are both pros and cons to use high and low trophic level species. However, they should fulfill some common requirements, e.g. sufficient amount, large geographical distribution and easy to collect. In term of the second category program, sport fish is commonly used as they are consumed by humans, especially humans living close to areas with rich water resources. Recently, over ten common fish species from Dianshan lake were screened for OHCs, and some of them (e.g. Yellow catfish) would be selected as prioritized species for monitoring (Unpublished). In Paper V, eggs of black-crowned night heron were analyzed for PCDD/Fs and related compounds. As such matrices are also consumed by local citizens, it is reasonable to include bird eggs in the second category of monitoring programs in the YRD.

As discussed in Chapter 3.1, pooling is an alternative to individual analysis in some cases (e.g. saving cost, accumulating target compounds). In Paper VI, pooling strategy was compared with individual analysis and the results showed that pooling, it can save cost (more collected specimens but fewer chemical analyses) while keeping the statistical power for temporal trend analysis at the same or even higher level as with individual analyses). However, pooling is not recommended for the second category program because it is necessary to investigate variability and outlier in the individuals to provide a conservative measure of overall risk.
Nevertheless, these two categories are not entirely independent. For instance, monitoring of chemical exposure to biological samples is relevant also for humans because trends in wildlife can often be assumed to be in parallel with trends in human food.

4.3.3 Sampling strategy (Paper VI)

Field sampling is the first step in the monitoring work, before effect and chemical analysis can be performed. Field sampling is costly, time and labor consuming so it is critically important to plan the monitoring effort in both detail and at depth. Regular sampling from a certain site is the most convenient way, but it is a question if the selected site is representative for the study area. In Paper VI, three sampling strategies were explored with computer simulations: Taking samples from (1) a fixed sampling site each year; (2) all samples from an arbitrary selected sampling site each year; (3) all sampling sites every year. The scenarios were compared by estimation of required sample size, to reach a detection of an annual change of 5% with a statistical power of 80% and 90% and significant level of 5%. The variance of the data was based on real analytical results (BDE-47) in snails from five sites in Tianmu lake.

The results showed that the second strategy (an arbitrary selected sampling site) is the worst strategy. To assure 80% power the sample size needs to be 2 or 3 times larger compared to strategy 1 and about 2 times compared to strategy 3. 90 % power were not reach even at sample sizes above 500 specimens. Also the number of overestimated trends was considerable compared to strategy 1 and 3.

The simulation results showed that the first strategy (a fixed sampling site) requires lower sample sizes than the third strategy (all sampling sites). However, the first strategy is sensitive to the condition of the sampling site. It commonly occurs in China that one site undergoes rapid development/change within a short period of time, and therefore, a fixed sampling site is not recommended for a long monitoring program. Another shortage of the first strategy, as mentioned previously, is the representativeness of the chosen study area.

To achieve a power of 80%, the third strategy required a moderate sample size. Although requiring a somewhat larger sampling effort this strategy is more robust and representative of the study area and is thus the recommended choice. A combination with pooled samples could further lower the analytical costs (see pooling discussion above).
5. Concluding remarks

As a Chinese, I have been taught since childhood that our nation has a glorious history. On culture, the pre-Qin thinkers with hundred schools of thought contend (770 BC-221 BC) have a great influence until now. On science and technology, the Great Four innovations (c.f. Chapter 1) make life much convenient. In recent forty years, the Chinese Miracle has happened, making our country gradually return to the center of the world. However, as the price of rapid economic development, the environment is getting worse and worse, i.e. anthropogenic chemicals threatening human health and the environment. Today, China is called “World Factory”, as supported by the findings in this thesis. Here I have picked out some thoughts from each of the papers I-VI.

In Paper I, mussel and clam were selected to get a general knowledge about OHCs contamination in coastal line area in China, and compared with other published results (e.g. “Mussel Watch” program). It clearly shows a severe pollution situation in terms of DDTs. In addition, HBCDD was detected at a comparable concentration as PBDEs, supporting the inclusion of HBCDD in monitoring programs. This may imply the importance of efforts on analysis of other flame retardant in general, and brominated in particular.

In Paper II, a refined method was developed for dicofol analysis, which is a suspected major source of DDT in China. Accordingly it is reasonable to pursue dicofol analysis which indeed is a challenge. However, it is possible to carry out analysis, in parallel to dicofol assessments, of the major dicofol decomposition product, DCBP. Environmental exposure to dicofol still needs to be confirmed. Unfortunately, too little data are yet available on this environmental contaminant. The method presented in this thesis needs further improvement to assure proper assessments of dicofol and DCBP in real environmental sample.

In Paper III, extensive OHCs contamination in various wildlife species collected from a paddy field near an industrial park is reported. Among them, the most important results were the very high wildlife concentrations of CPs. For the future, analysis of CPs must become a prioritized issue, especially in China, which is the largest producer and consumer of CPs in the world. The data obtained indicate Chinese CPs as more complicated than previously known commercial products. In depth studies must be promoted.
Further, a yet non-confirmed set of polychlorinated chemical congeners reported in Paper III must be structurally identified. This will require synthesis of authentic reference standards which has not yet been done. This is urgently needed. Likewise, further environmental occurrence assessments and (eco-) toxicity testing of these chemicals are required along with efforts to determine these chemicals in humans.

A novel pattern of OND-PCBs was observed in several wildlife species (Paper III) and with emphasis on eggs from black-crowned night heron and whiskered terns (Paper IV). These OND-PCBs may have some different sources, but it is worth to stress that there is a likelihood that they are byproducts from some ongoing pigment production, a source that is not well-known to the world. The occurrence of these OND-PCBs in wildlife needs much more attention calling for further monitoring efforts and confirmation of their sources.

In Paper V, an extension and in depth study performed on eggs from black-crowned night heron and whiskered terns reported in Paper IV. Emphasis in Paper V is put on dioxin content, PCP and PCP related contaminants. OCDD is clearly the dominating dioxin congener of PCDD/Fs with strong association to PCP as the source. PCP impurities and byproducts reported are several highly chlorinated congeners of PCDEs, OH-PCDEs and MeO-PCDEs, here reported, to the best of my knowledge, for the first time in biological samples from China. Interpretation of PCDD/F sources from a unique perspective was proposed by analyzing its precursors (e.g. PCDEs, OH/MeO-PCDEs) and assessing the correlation among them. PCP technical product and/or environmental samples taken from PCP manufacturing sites are of interest for future studies. However, the results reported in Paper V strongly call for monitoring efforts regarding dioxins in eggs for human consumption since the TEQ levels may be very high.

In paper VI, statistical power was introduced to evaluate various sampling strategies, to assist the design of long-term monitoring program of sedentary species (Paper VI). It strongly recommends sampling covering a larger part of the study area (and to avoid taking all the specimens from an arbitrarily selected spot).

Finally, this thesis improves the knowledge on environmental pollution situation of emerging and legacy OHCs in the YRD, by using wildlife as indicators. It reveals the importance and urgency to establish research monitoring programs of biological samples which not only learns from the existing experience, but also fits the regional situation. The data shown in this thesis urge for environmental monitoring efforts of OHCs in humans active in the YRD and to link such data to their health and wellbeing.
Det har skett en snabb ekonomisk utveckling i Kina under de senaste decennierna, särskilt i den region som benämns ”Yangtze River Delta (YRD)” i vilken flera stora städer ligger bl.a. Shanghai. Regionens urbanisering, industrialisering, jord- och vattenbruk har resulterat i omfattande produktion och användning av kemikalier i bred bemärkelse. Dessa kemikalier utgör ett potentiellt hot mot ekosystemens och människors hälsa men forskningen om kemikalier, hälsa och miljö begränsad i regionen.

Målet med denna avhandling är att bedöma miljöföroreningsnivån, miljögiftsmönster och källor till organiska halogenerade miljögifter (OHCs) i det låglänta området förhållandevis nära utloppet av Yangtze-floden. Djur från olika livsmiljöer används för att beskriva miljöförorenings-situationen i syfte att utveckla en avancerad och långsiktig metod för att övervaka miljögiftstrender i YRD men också applicerbart i hela Kina. Vidare har en robust metod utvecklats för att analysera bekämpningsmedlet, dikofol. En särskild insats har gjorts för att undersöka effekten med avseende på statistisk styrka av olika provtagningsstrategier speciellt för användning vid tidsserieanalys av stationära arter inom miljögiftsövervakningen.

Resultaten som redovisas i avhandlingen pekar på en mycket omfattande föröreningssgrad av de OHC som analyserats i biologiska prov från den vilda fauna. Särskilt anmärkningsvärt är de mycket höga koncentrationer som uppmätts av klorparaffiner (CPs) i en rad olika djurarter, inkl. en orm (Gloydius brevicaudus) och pilgrimsfalk (Falco peregrinus). Föroreningar i tekniska produkter av pentaklorfenol (PCP) eller transformationsprodukter av PCP har identifierats. Dessa utgörs av bl.a. polyklorerade difenyletrar (PCDEs) och hydroxylerade polyklorerade difenyletrar (OH-PCDEs) som påvisats och identifierats för första gången i ägg från natthäger (Nycticorax nycticorax) och skäggtärna (Chlidonias hybrida). Vidare påvisades höga koncentrationer av oktaklorodibenzo-p-dioxin (OCDD) i äggen från de båda fågelarterna. Summan toxiska ekvivalenter (TEQ) av polyklorerade dibenso-p-dioxin (PCDD) och polyklorerade dibensofuranner (PCDF) visas ligga på medelvärden av 520 och 300 pg TEQ g-fett i ägg från skäggtärna respektive natthäger. Detta är mer än två tiopotenser högre än Europeiska unionen (EU) gränsvärde på 2.5 pg TEQ g fett och oroande då åtminstone ägg från natthäger används av människor i regionen som ett livsmedel. Studierna redovisar
vidare på förekomsten av de mest högklorerade bifenylen som finns, bland den studerade vilda faunan. Dessa utgörs av okta-dekaklorerade bifenyler och benämns OND-PCBs i denna avhandling. Dessa PCBer bidrar till så mycket som 20% av den totala mängden PCB i ett antal djurarter. Förekomst och halter av en lång rad traditionella persistenta organiska miljöföroringar (POPs) redovisas i avhandlingen med DDT som det dominerande klororganiska bekämpningsmedlet. Även hexaklorcyklohexaner och mirex rapporteras i avhandling, vilket tyder på historisk användning av dessa bekämpningsmedel. Bland de kemikalier som används i tekniska sammanhang, PCB och de bromerade difenyletrarna (PBDE), är halterna förhållandevis låga i studiematerialet.

En förfinad och förbättrad analysmetod har utvecklats för att separera dicofol från sin dominerande nedbrytnings produkt, 4,4’-diklorbensofenon. Metoden medger analys och kvantifiering av dicofol som sådan.

En statistisk power-analys visar att provtagning av stationära organismer, såsom snäckor, bör insamlas på ett enhetligt sätt från ett antal olika provtagningspunkter spridda över det område som ska representeras för att ge de bästa och säkraste tidstrendsresultaten.

Resultaten som presenteras i denna avhandling visar mycket höga halter av klorparaffiner i en rad biologiska prov och likaså höga koncentrationer av perklorerad dibensodioxin (OCDD) i fågelägg från skäggträden och natthäger. Nivåer och mönster av OHCs i det studerade låglandsområdet vid Yantzeflodens utlopp skiljer sig från andra väl studerade områden i världen. Detta beror sannolikt på omfattande produktion och användning av kemikalier i YRD. Resultaten signalerar starkt behov av forskning om biologisk miljöövervakning. Om sådana program kan etableras finns en förutsättning för bättre hanteringen av kemikalier i YRD, med potential att expandera till utvecklingen av ett mera hållbart kinesiskt samhälle.
中文概要

迈入二十一世纪，长三角地区已成为举世瞩目的金融中心。不同于其它经济发达地区，这是一个仅用时二十多年就让全世界由“不了解”到“充满吸引力”的神奇区域。然而城市化和工业化在推动着区域快速发展的同时，污染治理措施和相关标准的制定却未跟上其脚步，使区域环境面临前所未有的压力。很难想象，这个被誉为“鱼米之乡”的富庶区域，如今逐渐面临“有水皆浑，有土皆污”的窘境。我们日常生活的环境、呼吸的空气、饮用的水和摄入的食物都面临着化学品的威胁。

持久性有机污染物（POPs）因具有持久性、生物富集性、长距离迁移性和生物毒性而受到广泛关注。2001年，经联合国环境规划署（UNEP）牵线，100多个国家在瑞典首都斯德哥尔摩签署了《关于持久性有机污染物的斯德哥尔摩公约》，旨在禁止或限制POPs的生产和使用。该公约自2004年起生效，起初共有12类化学品被列入POPs名单。如今，该名单已经增至26类。尽管如此，仍然有很多结构功能类似的新兴卤代有机污染物未在POPs名单中，但其生态毒性已受到科学界的广泛关注。

本论文对POPs及部分新兴卤代有机污染物在长三角区域典型环境中的污染水平、模式和来源进行了初步研究。野生动物（如贝类、鸟蛋）被用作评估环境污染的指示生物。此外，本论文对化学分析及环境监测的方法开发上也有所涉及，重点关注三氯杀螨醇（Dicofol）以及利用效能检验（power analysis）的方法协助设计长期的环境监测项目。

研究发现，传统有机氯农药（如DDT）污染较为严重；而如多氯联苯（PCBs）和多溴联苯醚（PBDEs）等工业品POPs，其污染浓度与西方国家和地区相比不算严重；二恶英类物质（PCDD/Fs）以高氯同系物或同分异构体为主，可能与历史上五氯酚作为防治血吸虫病的大量使用有关。对于新兴有机污染物，首先，在工业园区周围的生物样品中检测出高浓度的氯化石蜡；其次，五氯酚工业品中的杂质，如多氯联苯醚（PCDEs）、羟基多氯联苯醚（OH-PCDEs），首次在中国生物样品
中被发现，该类物质可考虑用作二恶英污染的预警标志物；再者，发现高氯代 PCBs 在生物样品中存在，其来源尚不明确。

研究表明，POPs 及新型卤代有机物在各类生物样品中被广泛地检测出。氯化石蜡、DDT 和二恶英等部分污染物的含量高于全球其它地区。履行 POPs 公约，既是机遇又是挑战。与发达国家相比，我国的基础研究数据不足，环境法规尚未健全。因此亟需建立适合国情并针对该类物质的长期环境监测项目，其意义不仅在于保护生态环境及人体健康，同时协助权威部门针对化学品的控制管理提供科学依据。
Acknowledgement

After an intensive marathon work, in the end the last part of my thesis has been arrived. The thesis, in parallel with four manuscripts runs from winter to the summer of 2016. Sitting at the campus in the lovely season, a myriad of thoughts came to my mind as there are so many people helping me physically and mentally during this journey.

I would like to express my deepest gratitude to my supervisor Åke Bergman for providing me an opportunity to get the insight of environmental chemistry. Thanks for your encouragement, enthusiasm and patient throughout the journey. You also set an example as how to be a group leader, project manager and scientist. I was impressed by your great effort to remember each name of Chinese person and location even though you still name me in your own way 😊

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I would like to thank colleagues from MMK and ACES, but outside from Miljökemi. Particularly **Zhe Li**, for all your help and support from Umeå to Stockholm; **Bo Yuan**, for your insights to CPs; **James Shen**, for talking with Shanghai dialect; and **Daqing Cui**, for your creative poetry.

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Miao, Yulan Jin, Yi Ni (Junior middle school), Guiying Zhong, Dibo Gan, Yanjun Zhang, Qihe Tao (Primary school) ….

Finally, my deepest gratitude to my parents for the unwavering love. 爸爸妈妈，谢谢你们无私的付出，支持我的决定。Also thank my aunt and uncle for providing excellent education in middle school, 特别谢谢姑姑自我读书开始就照顾卧床的奶奶，无论寒暑，无论风雨。My wife, Yihui Zhou (again), thanks for coming to my life with endless love, 谢谢我们彼此陪伴对方度过生命中漫长的寒冬。

I would also show my love to Stockholm, a lovely city who witnesses the fruitful journey for me during the past six years. It is my second home town.

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Appendix

Table A1. Detail information on homologous profile of polychlorinated biphenyls (PCBs) in Arochlor series products.

Table A2. Numbering system for PCBs, PBDEs and PCDEs according to Ballschmiter.

Table A3. Homologous profile of polybrominated diphenyl ethers (PBDEs) in commercial product.
<table>
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<th>Molecular formula</th>
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<th>Aroclor 1232</th>
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<th>Aroclor 1254(^b)</th>
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\(^a\) Lot A4 Aroclor 1254 (Monsanto Lot K102-6024) from abnormal late production (1974–1977)

\(^b\) Lot G4 Aroclor 1254 (GE/118-peak analytical standard)
Table A2. Numbering system for PCBs, PBDEs and PCDEs according to Ballschmiter.59

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Table A3. Homologous profile of polybrominated diphenyl ethers (PBDEs) in commercial product.65

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<th>Homologous (n)</th>
<th>IUPAC number</th>
<th>PentaBDE mixture</th>
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<td>Hexa-CDEs (42)</td>
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