Chemical Pollution Challenges in the Yangtze River Delta: Communication Brief
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长三角地区化学品污染挑战：经验与启示
Executive summary

Based on the cooperation, detailed results (c.f. Chapter 2, 3 and 4) and discussions from the research project – Chemstrres – we want to summarize our conclusions in a set of “Key Messages” and also pointing out our “Future needs”

The Key Messages are that: Chemstrres
- has brought scientists from China and Sweden together, to approach the global environmental chemical pollution threat in a coordinated manner to promote the management of hazardous chemicals.
- has created the first systematic study on environmental exposure of some high concern chemicals in Yangtze River Delta (YRD), including surface water, wildlife, sewage sludge, drinking water, indoor dust and human milk.
- has shown that chlorinated paraffins (CPs) are extensively distributed in wildlife and humans in the Yangtze river delta area, also from sources far away from the manufacturing sites, e.g. in northern Europe.
- has significantly improved our understanding of the bioaccumulative characteristics of CPs, showing that not only the occurrence of short chain but also medium and long chain CPs in biota is problematic.
- has initiated a continuous environmental monitoring program in Yangtze river delta area, including the build-up of the Yangtze Environmental Specimen Bank (YESB), enabling future evaluation of the effectiveness of bans and restrictions of chemicals.

Future needs
- Research need to be intensified in all the three areas of “One Health”, i.e. environmental, wildlife and human health, to close up on Chemical safety in China, Sweden and globally.
- The risk of combined exposures to mixtures of chemicals must be addressed.
- Research related to chemicals, environment and health requires international collaboration.
- It is a need to establish a long-term program with guaranties for financial support of the Yangtze Environmental Specimen Bank (YESB).
- Intensified health and wildlife risk research of prioritized chemicals is needed, firsthand on chlorinated paraffins and on their toxicity.
- Further development of joint efforts in learning and management are needed to move away from “in silo” thinking and actions.

The subprojects carried out (Chapters 3.1 – 3.10) are giving more insights to specific issues of concern and possibilities. Therefore, it is important to scrutinize these chapters and even more, read and comprehend the around 50 scientific, peer reviewed articles that have emerged from Chemstrres.
Contents

1. Introduction with Main Conclusions ................................................................. 8

2. Building for the future ........................................................................................... 16
   2.1 Building the Jiaxing laboratory ........................................................................... 17
   2.2 Building and creating the Yangtze Environmental Specimen Bank ..................... 20
   2.3 The collaborative achievements through Chemstrres ....................................... 28

3. Chemstrres research: Main results and messages .............................................. 34
   3.1 Ecotox project with the pond snail (*Bellamya aeruginosa*) ............................... 35
   3.2 Ecotoxicology of chlorinated paraffins ............................................................. 41
   3.3 Establishment of *Bellamya aeruginosa* as a research monitoring species in the Yangtze River Delta .................................................. 45
   3.4 Drinking water contaminants – a screening project .......................................... 50
   3.5 Sewage sludge as a mirror of human activities ................................................. 55
   3.6 Assessing persistent and bioaccumulative compounds in wildlife from the Yangtze River Delta ......................................................... 60
   3.7 Heron eggs and environmental quality in the YRD region .................................. 67
   3.8 Mothers’ milk monitoring in the Yangtze River Delta ....................................... 74
   3.9 Establishment of optimal fish species for research monitoring in the Yangtze River Delta ................................................................. 78
   3.10 Chemical pollutants in dust ............................................................................. 83

4. Scientific output and basis of Chemstrres ...................................................... 90
   4.1 Publications and Doctoral thesis from Chemstrres (alphabetical order) ............... 91
   4.2 Principal Investigators and Contributing scientists ............................................ 95
   4.3 Chemstrres reference group ............................................................................. 97

Acknowledgement .................................................................................................. 98
Chapter 1:

Introduction with Main Conclusions
The project “Swedish-Chinese chemical pollution stress and risks research program in the Yangtze River Delta region” with the acronym “Chemstrres” started 2014 with continuous funding until the end of 2019. The Swedish Research Councils VR, Formas and Forte invested in the project to improve knowledge transfer from China to Sweden and vice versa. The project partners are scientists from College of Environmental Science and Technology, at Tongji University in Shanghai and Jiaxing, the Swedish Museum of Natural History in Stockholm and Department of Environmental Science and Analytical Chemistry at Stockholm University.

Chemstrres was aimed to develop a structured framework for assessment of chemical environmental hazards and risks in the highly industrialized and trade intensive Yangtze river delta, where the commercial center and megacity, Shanghai, is located. The region also incorporates extensive agriculture and aquaculture. The goal was to strengthen Swedish and international research on hazard identification and risk assessment by working in a heavily contaminated region, with implications for human health, particularly via contaminating food and drinking water. The extensive trade of products, materials and goods from the YRD region impinges on the potential contamination of the environment worldwide. Chemstrres was further aimed to support management of chemicals and the environment, both in China and in the EU/Sweden. The proposed framework was envisioned to be applicable for environmental contamination risk assessment worldwide.

Chemicals distributed via products, materials and goods have global distributions due to the extensive global trade. Food for export may be similarly distributed and contaminants distributed locally will find their way far from the sources when persistent enough. It is of importance that societies are given the opportunity to react to; manage the chemical pollution threats.
Chapter 1: Introduction with Main Conclusions

To reach these goals, a framework for development and integration of interdisciplinary cooperation for assessment of the environment, wildlife and food/water quality was proposed. The framework innovatively integrates:

– Study design, methods and evaluation;
– Environmental exposure assessment and
– Ecotoxicological effect assessment, surrounding the central issue of improved hazard and risk assessments of chemicals and the environment.

The aims of Chemstrres can be visualized as shown in Figure 1.1.

The project has been carried out in two main directions, Research and Learning & Outreach. This is described in Figure 1.2 and further described as follows.

**Research:** Based on close interactions and visits by the Chinese professionals to Stockholm and with the Swedish researchers visiting Shanghai/Jiaxing promoted the development into ten different subprojects. Important research was carried out at all of the partner home institutions, with most important sampling activities in the YRD but also in Sweden. There is a subdivision in the research section dealing with study design, methods and evaluation and monitoring efforts. The former is critically important for the latter and need to be taken into serious discussions in order to manage high quality and long lasting monitoring of pollutants and evaluation of trends.

The outline of the Chemstrres subproject research activities is presented in Figure 1.3.

![Chemstrres project outline](image)
The ten subprojects were described in the previous Chemstrres book (2017), Chapter 3, and the initial results were also addressed for two of the subprojects in Chapter 4, related to 3.3 and 3.6/3.7. Main results of the subproject and subproject key messages and implications are presented for each of the projects visualised in Figure 1.3 and listed below.

3.1 Ecotox project with the pond snail (*Bellamya aeruginosa*)
3.2 Ecotoxicology of chlorinated paraffins
3.3 Establishment of *Bellamya aeruginosa* as a research monitoring species in the Yangtze River Delta
3.4 Drinking water contaminants – a screening project
3.5 Sewage sludge as a mirror of human activities
3.6 Assessing persistent and bioaccumulative compounds in wildlife from the Yangtze River Delta
3.7 Heron eggs and environmental quality in the Yangtze River Delta region
3.8 Mothers’ milk monitoring in the Yangtze River Delta
3.9 Establishment of optimal fish species for research monitoring in the Yangtze River Delta
3.10 Chemical pollutants in dust

**Learning and Outreach:** Early personal contacts and interest from both sides, Tongji University researchers and students brought us together as a start of educational exchange. A first few students took the Master exam in Environmental Chemistry at Umeå together with Stockholm University. Several Master theses were presented on work done at Stockholm University. This actually started prior to the Chemstrres project, under a project financially supported by the Swedish International Development
Figure 1.3. The Chemstrres ten subprojects, 3.1 – 3.10, graphically introduced here are presented with their full titles on the previous page.
Cooperation Agency (SIDA) and right after a Memorandum of Understanding (MOU) was signed in 2010. This had been preceded by professor Yanling Qiu working as a guest researcher together with the professors Anders Bignert and Åke Bergman.

The exchange of students and some other visits were instrumental for launching the Chemstrres project. Now it was mandatory to get Chinese professionals from Tongji University to Stockholm for training, interaction and to perform research. That actually became the start of the exchange since the Swedish partner spent time at Tongji University, participating in project planning, sampling and training. The key to success was the annual Sino-Swedish Workshops that were held every second year in Shanghai and Stockholm, respectively.

Chemstrres invited a high ranked Reference group (see chapter 4.3) with representatives from both China and Sweden. The project communication to and input from the Reference group have been of very high value, both for societal interaction and scientific reasons.
Chapter 1: Introduction with Main Conclusions

The participant gathered at the Final Swedish conference of Chemstrres, February 8, 2019.

Overarching conclusions (Key messages) and future needs

Chemstrres:
- has brought scientists from China and Sweden together, to approach the global environmental chemical pollution threat in a coordinated manner to promote the management of hazardous chemicals.
- has created the first systematic study on environmental exposure of some high concern chemicals in Yangtze River Delta (YRD), including surface water, wildlife, sewage sludge, drinking water, indoor dust and human milk.
- has shown that chlorinated paraffins (CPs) are extensively distributed in wildlife and humans in the Yangtze river delta area, also from sources far away from the manufacturing sites, e.g. in northern Europe.
- has significantly improved our understanding of the bioaccumulative characteristics of CPs, showing that not only the occurrence of short chain but also medium and long chain CPs in biota is problematic.
- has initiated a continuous environmental monitoring program in Yangtze river delta area, including the build-up of the Yangtze Environmental Specimen Bank (YESB), enabling future evaluation of the effectiveness of bans and restrictions of chemicals.
The Chemstrres project participants gathered energy prior to the start of the Final workshop in Tällberg, Sweden in February 2019.

**Future needs**

- Research need to be intensified in all the three areas of “One Health”, i.e. environmental, wildlife and human health, to close up on Chemical safety in China, Sweden and globally.
- The risk of combined exposures to mixtures of chemicals must be addressed.
- Research related to chemicals, environment and health requires international collaboration.
- It is a need to establish a long-term program with guaranties for financial support of the Yangtze Environmental Specimen Bank (YESB).
- Intensified health and wildlife risk research of prioritized chemicals is needed, firsthand on chlorinated paraffins and on their toxicity.
- Further development of joint efforts in learning and management are needed to move away from “in silo” thinking and actions.
Chapter 2:

Building for the future
The Tongji University scientists have created a foundation for the future by focussing on the construction the Jiaxing laboratory (2.1) and the Yangtze Environmental specimen bank (2.2). Together the Chemstrres scientists have managed to bring these investments into operation. Further, the interactions between the Swedish and Chinese researchers have led to significant achievements which is elaborated on in this chapter, and chapter 3 and 4.1.

2.1 Building the Jiaxing laboratory

In 2010, the 1st Sino-Swedish Workshop was held in Stockholm with the main objective to develop a monitoring program of chemical pollution around the Yangtze River Delta, one of the most developed regions in China. Two facilities were included in the monitoring program, facilities that required to be built. One is the Jiaxing laboratory or as also called, the Sino-Swedish Environmental and Health Laboratory (SINOSWEEHL), and the other is the Yangtze Environmental Specimen Bank (YESB). The Jiaxing laboratory and the YESB have been established in the Sustainable Park in Jiaxing city, in the central part of the Yangtze River Delta. Jiaxing holds an advantageous geographic location, with Lake Tai in the north, and just about a hundred kilometres (62 miles) from Shanghai, Suzhou and Hangzhou. The facilities in the Jiaxing Sustainable Park are shown in the photo (Figure 2.1.1).

Fig 2.1.1 The Sustainable Park and the Jiaxing-Tongji Environmental Research Institute, also known as the SINOSWEEHL.
Chapter 2: Building for the future

Sino-Swedish Environmental and Health Laboratory

The Sustainable Park has an area of 100,000 m², where the Jiaxing-Tongji Environmental Research Institute has two buildings (i.e., experimental and training building) and one underground construction, i.e., the YESB. The layout of the Lab is shown in Figure 2.1.2, with 2000 m² for sample pre-treatment and instrument analysis, including rooms for sample preparation, storage, washing, heating, weighing, organic analysis pre-treatment, inorganic analysis pre-treatment, instrumental analysis, and cleanroom areas.

The interior of the sample preparation room can be seen in Figure 2.1.3. Serval instruments, such as total organic carbon analyser, gas chromatography-mass spectrometer, microscopic analysis, are shown Figures 2.1.3. Based on these facilities, we can analyse the concentrations of organic and inorganic chemicals in environmental samples. In addition, the hazards of pollutants on environmental organisms and human health and also the underlying mechanism can be examined in the laboratories.
Chapter 2: Building for the future

The pre-treatment room for organic analysis

Total organic carbon analyser.

Gas chromatography-mass spectrometry.

Gas chromatography-tandem mass spectrometry.

Microscopic analyser.

**Figure 2.1.3.** Photos from inside of the Jiaxing Laboratory
2.2 Building and creating the Yangtze Environmental Specimen Bank

Brief introduction
The Yangtze Environmental Specimen Bank was initiated by Tongji University in 2010, under competent support from the colleges at Stockholm University and Swedish Museum of Natural History. Jointly supported by the State Key Laboratory of Pollution Control and Resource Reuse and the Key Laboratory of the Yangtze River Water Environment, Ministry of Education, the YESB aims to collect, prepare, and store on a long-term basis, environmental samples taken from the Yangtze River Delta and the Yangtze River Basin. In 2011, the small-scale YESB was initially operated in the South campus of Tongji University and the large-scale YESB was built at the Sustainable Park in Jiaxing City, Zhejiang Province in 2013, consisting of preparation room, cool room, refrigerator room, scientific demonstration room, information management system, and analysis lab, with a total area of 3000 m². Currently, over 20,000 individual samples are stored in the YESB, including soil, sediment, sewage sludge, fish, mussel, bird egg, human hair and breast milk. Researches have been involved in the preparation of samples and analysis for pursuing studies of temporal and spatial distribution fate and toxicity. Further, also to study human risk of pollutants in the various environmental matrices, which provide basic data for the risk assessment and in a longer run, management of chemicals produced and consumed in the society. In addition, the YESB is playing an important role in global ESB network and social education on environment. In the near future, as a platform of science and technology, the YESB can provide more support for the eco-friendly and integrated development of the Yangtze River Delta and the eco-environmental protection covering the Yangtze River Economic Belt.

The history of the YESB
The Environmental Specimen Bank (ESB) is a system for the systematic collection and long-term storage of specimens, which has been established since the 1970’s in developed counties and recognized as a fundamental complement for environmental monitoring and scientific research. Currently, the
value of ESB is becoming more broadly recognized globally, while China is still at the early stage of such a development.

- **2007-2008**: Prof. Yanling Qiu of Tongji University was a visiting scientist at the Department of Environmental Chemistry at Stockholm University, headed by Prof. Åke Bergman. The one-year visit included collaboration also with Prof. Anders Bignert at the Swedish Museum of Natural History. Prof. Qiu recognized the importance of long-term environmental monitoring during her visit which became the starting point for further development at Tongji University.

- **2009**: Professors Bergman and Bignert payed their first visit to Shanghai and Tongji University to learn and make further contacts with scientists at the university, and to introduce the environmental monitoring research in Sweden and the ESBs in Sweden and worldwide.

- **2010**: Prof. Jianfu Zhao, Tongji University proposed to develop a Memorandum of Understanding (MOU) between Stockholm University, the Swedish Museum of Natural History and Tongji University. As mentioned above (Chapter 1) the MOU was signed on September 8. The aim of the MOU included examination of the risk of anthropogenic chemicals to the environment and human health. One of the main tasks of the MOU was to establish the YESB.

- **2011**: The YESB started at the South campus of Tongji University, with a volunteer team to help in the collection and preparation of environmental samples for the specimen bank.

Also, Tongji University and Jiaxing City reached an agreement on the development of the Jiaxing-Tongji Environmental Research Institute, including the collaboration on the development of the YESB.

- **2013**: The YESB organized the International Conference on Environmental Specimen Banks in Shanghai, with the objective to provide a focus discussion on the sustainable development, management, and international cooperation of Environmental Specimen Bank network coping with regional and global environmental changes. More than a hundred scientists from more than ten countries joined in the conference.

- **2014**: The YESB became a member of ESB network. Prof. Anders Bignert gave a series lectures and training courses for young researchers of the YESB on sampling strategy, practices in field sampling work and statistical analysis in environmental monitoring.

- **2015**: Prof. Jianfu Zhao and Prof. Xiang-Zhou Meng of the YESB and Prof. Paul Becker from Hollings Marine Laboratory of the United States, as guest editors, organized a Special Issue entitled “Developments and Applications of Environmental Specimen Banks for Monitoring Emerging Contaminants (ECs)” that was published in *Environmental Science and Pollution Research* (2015, Volume 22, Issue 3). Overall, 31 papers address the latest development of ESB and the scientific advancements on emerging contaminants in the environment.

- **2016**: The cool room and the sample information system of the YESB went into operation.
– **2017**: In the 3rd Committee members of the Key Laboratory of the Yangtze River Water Environment, Ministry of Education, Prof. Jianfu Zhao and Prof. Daqing Yin proposed that the YESB should be developed as a featured platform during the first session, which should promote long-term achievements. The Jiaxing-Tongji Environmental Research Institute (where the YESB is located) was honored as Zhejiang Ecological Education Base.

– **2018**: The 3rd Committee members of the Key Laboratory of the Yangtze River Water Environment, Ministry of Education proposed that the YESB should pay more attention on analytical method development during the second session, which provides scientific support for the eco-environmental protection covering the Yangtze River Economic Belt. The scientific demonstration room opened and the Jiaxing-Tongji Environmental Research Institute was honored as Zhejiang Ecological Education Base.

– **2019**: The YESB joined the Protection Program of the Yangtze River, and two technicians became involved in the public science education and engaged in collection of samples along the Yangtze River. The YESB was selected as a component in the video entitled “Love in the Yangtze River”, showing its importance in the protection of the River.

The main entrance of the YESB in the Sustainable Park, Jiaxing.
Chapter 2: Building for the future

The YESB cold storage room.

The refrigerator room.

The specimen pre-treatment room.

The scientific demonstration room.

**Figure 2.2.1.** Photos from the YESB facilities with one on top of the page to the left, in front of the entrance to the underground storage building.
The sample information and sample management system of the YESB

Currently, more than 20,000 individual environmental samples have been collected from the Yangtze River Delta and the Yangtze River Basin which are now stored in the YESB. The specimen includes soil, sediment, sewage sludge, fish, mussel, bird eggs, plant, human hair, and breast milk. The brief information of samples is summarized in Table 2.2.1.

The YESB developed several standard operation procedures (SOPs) and a system to manage all samples. Each sample is given a two-dimensional barcode, with information such as sampling time, location and collector. The barcode is very convenient to manage large numbers of samples (see Figure 2.2.2).

Research project and outcome of the YESB

Based on the YESB, several projects have been conducted, including two international collaboration projects, seven projects from the National Natural Science Foundation of China, and six province-level projects. The research contents covered the analysis method, distribution, pattern, fate, toxicity, and human exposure to chemicals. More than 50 articles have been published in well-known journal such as Environmental Science & Technology. In addition, one Special Issue was organized in Environmental Science and Pollution Research. More details can be found in the sub-chapters of this book (Chapter 3) and in the publication list, Chapter 4.1.

One important aspect of environmental specimen banking is that the ESB has a longer time perspective than a time limited study for a special purpose. To be able to build long time-series for future temporal trend assessments of hazardous chemicals and to have stored specimens, collected long ago, available also for new or improved analytical techniques (e.g. non-targeted analysis). It is imperative that studies of contaminants in environmental samples are carried out in a close and integrated collaboration with an ESB. The ESB is thus not only a cold store facility but a national infrastructure to assure consistent sampling and reliable well documented storage. The ESB should thus promote the development of sampling protocols that can assure also that quantitative aspects for e.g. statistical analysis are fulfilled. Unintended collection of interesting animals found dead can of course be stored in the ESB but, to defend the high costs of the maintenance of an ESB, its main purpose needs to focus on well-studied species collected at certain sampling locations over a long time, in sufficient amounts following thoughtful and well elaborated protocols.

The ESB staff should, together with the international ESB-network, develop knowledge and guidelines

<table>
<thead>
<tr>
<th>Sample species</th>
<th>Year of starting</th>
<th>Sample sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>2007</td>
<td>The Yangtze River Delta, China and polar areas</td>
</tr>
<tr>
<td>Sediment</td>
<td>2006</td>
<td>Lake, river, and ocean of China and polar areas</td>
</tr>
<tr>
<td>Sewage sludge</td>
<td>2010</td>
<td>Wastewater treatment plants in China (especially in the Yangtze River Delta)</td>
</tr>
<tr>
<td>Fish</td>
<td>2011</td>
<td>The Yangtze River Delta</td>
</tr>
<tr>
<td>Mussel</td>
<td>2013</td>
<td>The Yangtze River Delta</td>
</tr>
<tr>
<td>Bird eggs</td>
<td>2013</td>
<td>The Yangtze River Delta and the Upper Yangtze Basin</td>
</tr>
<tr>
<td>Plant</td>
<td>2011</td>
<td>The Yangtze River Delta</td>
</tr>
<tr>
<td>Human hair</td>
<td>2012</td>
<td>Children and adults in Shanghai</td>
</tr>
<tr>
<td>Breast milk</td>
<td>2015</td>
<td>Shanghai, Shaoxing, and Jiaxing cities</td>
</tr>
</tbody>
</table>
Concerning freezing techniques and effects on sample degradation (fat and contaminants of varying stability) during long time storage. The development of extremely clean laboratories for sample preparation of samples uncontaminated also from chemicals we do not consider as contaminants today.

Social services by the YESB
The YESB does social service, especially in public education in environmental protection. In recent years, Chinese central government and local governments have made several efforts on eco-environmental protection covering the Yangtze River Economic Belt and Garbage Classification. The results produced by the studies storing their samples in the YESB are kept in a database and the data can thus be retrieved for integrated future reports about the environmental status and challenges in the investigated areas, like Lake Tai, Tianmu Lake, and Chongming Island. In addition, we can present the purpose of the YESB to the society and students from high schools and universities, as shown by the photos in Figure 2.2.3.

The Green Ring Volunteer Association is a non-profit organization serving the YESB since 2011. The team mainly engage undergraduate students in the college of Environmental Science and Engineering at the Tongji University. The Green Ring Volunteer Association has carried out a large-scale soil sampling program in Shanghai (two developed regions, Chongming Island and Lingang of Nanhui District). Environmental samples were collected in hot spots sites like areas surrounding Jinshan Petrochemical factory and Pudong Kangqiao area. The volunteers also participated in various environmental research related to the YESB. At present, the Association has successfully applied for the 6th environmental protection public welfare of the Chinese Environmental Protection Foundation, the China Soong Ching Ling Foundation, the Starbucks College Student Environmental Protection Project, the National College Student Innovation Experiment Project, and the Shanghai University Student Innovation Program Project. The volunteers gave lectures in more than ten primary and secondary schools such as Fukuyama Foreign Language Primary School.

Collaboration with the YESB
Since 2010, main researchers of the YESB have visited ESBs of Sweden, Germany, Japan, the United States and France, and our technicians were educated through technical training in Sweden. The YESB has established close relationship with ESBs mentioned above and become an important member of the global ESB network. To promote fundamental research in the field of global environment, the “International Conference of Environmental Specimen Bank (ICESB)” are jointly organized approximately every two years. During the
Chapter 2: Building for the future

Postgraduates from Tongji University visiting the YESB.

High School students learning about chemical analysis.

Social practice for young people from the Nanhu District.

YESB Volunteers participate in public science education activities.

Figure 2.2.3. Outreach activities related to YESB.
preparation and construction of YESB, main researchers of the YESB also participated in the ICESB conferences held in Germany (Berlin 2010), China (Shanghai 2013, organizer), France (Nancy 2015), Sweden (Stockholm 2019), the Sixth World Congress on Environmental Toxicology and Chemistry in Germany (Berlin 2012), and the DIOXIN meetings in Australia (Queensland 2012), Poland (Krakow 2018) and Japan (Kyoto 2019), for more communication with other ESBs and research groups related to persistent organic pollutants.

The YESB has built the relationship with institutes inside and outside China. Since 2011, the workshop of Sino-Sweden Environmental and Health Risk on Environmental Pollutants has been held annually (cf. 2.3). In 2018, the Sino-Sweden International Cooperation Project Chemstrres aims to become an expert in environmental chemistry, toxicology, environmental management, and to set up a platform to conduct in-depth discussions on the environmental occurrence, exposure levels and ecological and health risks of human environmental pollutants, and make positive contributions to the promotion of human health.

In March 2015, Professor Jianfu Zhao (YESB project investigator) led the project of “Tongji University and World-Wide Fund for Nature (WWF) Cooperation on Promoting Sustainable Development of the Yangtze River Economic Belt (2015-2020)” in Tongji University. In May 2019, the Yangtze River Aquatic Bioremediation Promotion Project was sponsored by the World-Wide Fund for Nature (WWF), the Key Laboratory of the Yangtze River Water Environment, Education of Ministry, and the YESB.

The staff of the YESB participated in the popular science group to collect environmental samples along the river and accompanying science popularization activities. During the voyage, the Science Popularization Group received an interview with the “Life Changjiang” of Social and Legal Channel of China Central Television, "Save the Evil in the River" of China Agriculture Press, and "Love in Changjiang" of the Shanghai Television Station.

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Chapter 2: Building for the future

2.3 The collaborative achievements through Chemstrres

The collaborative interactions have been at several levels and on a multitude of tasks which makes it difficult to present a comprehensive review. Therefore, we present examples under the headlines below.

**Sino-Swedish Workshop on Environmental and Health Risk of Anthropogenic Environmental Pollutants**

This workshop series started already in 2010 and it has been possible to continue the meetings on an annual basis to allow all researchers to meet for exchange of information and both formal and informal discussions. The meetings were primarily held in Shanghai at Tongji University or in Jiaxing at the Jiaxing-Tongji Environmental Research Institute and at Stockholm University or the Swedish Museum of Natural History.

The workshop in 2014 was focused on which areas of environment and health research that should be prioritized for the Chemstrres project. The discussions were taken place at the Swedish Museum of Natural History and were indeed intensive and also constructive. The outcome of the workshop was a specification of nine subprojects (#1–9), presented both in the first Chemstrres book and in the present book, cf. Chapter 1 and 3. The 10th subproject was brought in during 2016 as we saw reasons to look much more closely on the exposure to pollutants on dust in our homes, both in Sweden and China. It was possible to create links and collaborations between the Chemstrres subproject #10 and EDC-2020 which has just presented their summary for policy-makers (www.swaccs.se ) and also the Misse project (https://www.aces.su.se/misse/), both funded by the Research Council, Formas. The ten subprojects were presented in the Chemstrres book that came out 2017 (http://su.diva-portal.org/smash/get/diva2:1138019/FULLTEXT01.pdf).

Each one of the forthcoming workshops included follow up on the projects but also presentations by scientists outside the Chemstrres consortium. The
workshop 2017 in Hangzhou focused on the finalization of the project.

The workshops included site visits, such as a visit to a modern Swedish drinking water plant supplying Stockholm with water from Lake Mälaren. At another workshop the wastewater treatment plant, Käppala (Lidingö), was visited and an extensive tour was offered by the plant management. The workshop 2016 was held in Visby at the Swedish island, Gotland, in the facilities run by Uppsala University. This time the field station, Ar, at the Northern part of the island was introduced to all the Chemstrres partners. When the workshop was held in Shanghai the participants were informed about the development of the Jiaxing laboratory and the environmental specimen bank and also made a visit in Hangzhou.

Visiting scientists to Stockholm as part of Chemstrres

In order to promote the exchange of competence, experiences and ideas and to fulfill the aim of the funding agency, the project applicants from Tongji University visited Sweden and Stockholm University for one to three months every summer during the period 2014-2018. The time was spent on discussions and knowledge exchange. The visit 2016 was devoted to work on the first Chemstrres book (Chemstrres 2017). In 2018, a hectic summer month was spent of discussions on planning the finalization of the project and the Final conference and workshop in February, 2019.

Valuable contacts were established with universities outside Stockholm University, first-hand the Swedish University of Agricultural Sciences (SLU) and Örebro university (ORU) with which MOU’s have been signed 2018 and 2019, respectively. Some initial contacts were also established with University of Gothenburg and Lund University during the duration of Chemstrres. The contacts have led to a 2019 November workshop in Shanghai to discuss future collaborations between researchers from Tongji University and from Sweden, first-hand linked to Swaccs (www.swaccs.se). These are scientists looking into all scientific aspects on chemicals, health and environment being convinced about the fact that we need to work together to meet the UN Sustainability Developmental Goals (SDGs). More than half of the
Chapter 2: Building for the future

Figure 2.3.2. Photos from the 7th Sino-Swedish Workshop on Environmental and Health Risk of Anthropogenic Environmental Pollutants was held 2016 in the old city of Visby, on Gotland, the biggest island in the Baltic Sea. The workshop participants also visited the fish research station Ar in the North West of the island.
SDGs are related to chemicals, health and environment. This is the background for pointing out the primary SDGs for each of the subprojects reported on in Chapter 3.

During the summer months the Chemstrres project group visited, in addition to Gotland, also Västmanland/Närke, Jämtland and Bohuslän, four provinces in Sweden to learn about environmentally related issues around Sweden. The visits were integrated with project planning.

The Chemstrres started with several longer stays of scientist at the Stockholm university laboratory to learn and work on chemical analysis of pollutants in wildlife, ecotoxicology and sampling. Master thesis projects were pursued, Ph.D. work and postdoc visits were part in the exchange during the whole project period. These collaborations led to systematic studies on environmental exposure of some high concern chemicals in Yangtze River Delta, to ecotoxicological achievements and methodological competence provision. The achievements are presented in Chapter 3 and the full publication list in Chapter 4.1

The Swedish partners were similarly given the options of a range of interesting meetings with business and industry, authorities and other universities. These contacts were valuable to build the base for future cooperation. The possibilities to present and discuss Chemstrres at national Chinese conferences made a great impact on the network for the Chemstrres scientists with professionals in China and Sweden. One of the tours was to Zibo in the Shandong province which included the visit to the wet land beside the Xiaofu River, the biggest local pharmaceutical company. The tour also brought us to Qingdao, where we visited Qingdao University of Technology and the Jiaozhou Bay. Better understanding of the environmental problems in other parts of China than the YRD was established through the site investigation and discussion with the local company, university and government.

Possibilities were given the Swedish partners to teach at Tongji University which have been appreciated from both sides. The contacts with students, in particular at the master and doctoral levels, have been of value.

Organisation of joint conferences and other scientific meetings
An important task in relation to improve collaborations have been to look for possibilities of co-organising larger meetings. This was accomplished by the following three larger meetings. In 2013, the International Conference on environmental specimen banks was held at Tongji University (October 2013) with some 100 participants, from China and outside. This conference has been discussed under Chapter 2.2.

United Nations Environmental Program (UNEP) took the lead and requested together with the World Health Organisation (WHO) a State of the science document on Endocrine disruptors ten years after the first one came out in 2002. Prof. Åke Bergman was appointed Principal Coordinator of the latter document but also responsible for parts already for the 2002 report. The document was launched in February 2013 (https://www.who.int/ceh/publications/endocrine/en/). Thereafter this status report was presented and discussed among most of the UN regions. However, it was not brought out in a corresponding manner in China. Therefore, it was a possibility for Tongji University to host a first workshop on this topic in China (2015) and then a follow up, three years later, in 2018. In the meantime, the full original report was translated into Chinese and is now available as such. The two workshops hosted several outstanding international experts on endocrine disruption.
Figure 2.3.3. Researcher within Chemstrres visited several parts of Sweden, in all directions from Stockholm. In the upper row (left), the province of Jämtland is shown when visited. Jämtland is located North-west of Stockholm, with mountains in the west, huge forest areas and wilderness; to the right prof. Jianfu Zhao is interviewed by the Local Swedish Radio in Örebro when visiting early sights for mining in Västmanland. In the lower row you can see two photos from the Swedish westcoast, to the left the research field station Svante Lovén Centre in Kristineberg and the photo to the right is a lighthouse in the outer part of the Swedish national park, Kosterhavet.
The two workshops were:

**2015**: 1st Chinese Open Workshop on Endocrine Disruptors – 2015 co-organized by Tongji University, China; Swedish Toxicology Sciences Research Center, Sweden; National Natural Science Foundation of China and China National Center for Food Safety Risk Assessment (October 2015) with approximately 35 participants with world experts attending from abroad. More details at Tongji News TV.

**2018**: 2nd Chinese Open Workshop on Endocrine Disruptors – 2018 co-organized by Tongji University, China; Swedish Toxicology Sciences Research Center, Sweden; National Natural Science Foundation of China and China National Center for Food Safety Risk Assessment (October 2018) with approximately 35 participants with world experts attending from abroad.
Chapter 3:

Chemstrres research: Main results and messages
3.1 Ecotox project with the pond snail (*Bellamya aeruginosa*)

**Objectives**
Clean water and sanitation are prerequisites for reaching sustainability. That will include life both under water and on land. In this project, caged pond snails *Bellamya aeruginosa* were employed to assess the sediment quality of the Taihu Lake. The objectives were as follows:

– to determine the suitability of a battery of biochemical biomarkers analyzed in caged *B. aeruginosa* after exposure to contaminated sediments under field conditions as a tool for the assessment of hazards posed by sediments.

– to identify applicable biomarkers for the battery.

– to evaluate the sediment quality of Taihu Lake involving both chemical analysis and toxic effects on basis of the biochemical biomarker battery.

**Main results**
As sinks and secondary sources of heavy metals and organic pollutants in the aquatic environment, sediments may pose direct threats to benthic biota and organisms. Routine chemical analyses and biological sediment toxicity assays are useful tools for the assessment of sediment quality. Toxicity tests have been proven to be highly useful and relevant as they can often be done more quickly and inexpensively compared to chemical analyses, and because they can provide insights into the potential hazard of sediment-bound pollutants. Field toxicity tests using caged organisms present several advantages over laboratory toxicity testing and indigenous community surveys. The use of caged organisms can minimize the influence of adaptive mechanisms, which may have evolved in resident organisms over time under long-term chronic exposure conditions, and that would lead to underestimation of pollution.
Chapter 3: Chemstrres research: Main results and messages

The pond snail *Bellamya aeruginosa* (Gastropoda, Prosobranchia, Valvatidae) is a dominant community member of freshwater aquatic systems in China. It is also a key species involved with the transfer of contaminants through the food web in Chinese surface waters, since it is used for human consumption and a primary food item of the black carp (*Mylopharyngodon piceus*). Therefore, it plays a key role in the assessment of contaminant risks to the aquatic ecosystems in China. Taihu Lake is the third largest freshwater lake in China and an important drinking water source for surrounding cities. In this project, the caged pond snail *B. aeruginosa* was used to assess the sediment quality of Taihu Lake.

In order to identify the appropriate biomarkers for the test battery, the initial work was related to identification and assessment of what to be applied in this context. Oxidative stress is commonly addressed in many studies since it is an essential physiological mechanism known to be affected by biotic and abiotic factors.

It is a process initiated by the imbalance between the production of oxidants and their removal by antioxidants and antioxidant enzymes. Reactive oxygen species (ROS), are produced during normal cellular respiration in mitochondria, or leaked

Figure 3.1.1. System used for *in situ* transplants of caged pond snails in Taihu Lake.
from enzymatic activity including the Phase I biotransformation enzyme cytochrome P4501A (CYP1A), and are normally metabolized by antioxidants. This includes antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxides (GPx) or molecular antioxidants like glutathione. The activity of EROD (ethoxyresorufin-O-deethylase) represents the activity of CYP1A. ROS levels are important in homeostasis, and can act as key regulator of biological processes. Generally, the excess of environmental pollutants accumulated in the living organisms can enhance the intracellular formation of ROS. The imbalance between production of ROS and antioxidant defenses may lead to oxidative stress manifested as oxidative damage of lipids and proteins. Changes in the concentration of antioxidants or oxidative damage products are often used as indicators of environmental stress and pollutant exposure. Hence, our selection of the biomarker battery includes the activities of EROD, SOD and CAT, ROS, as well as the protein carbonyl content (PCC) and lipid peroxidation (LPO).

In our study, the snail *Bellamya aeruginosa* was put in the cylindrical cages (0.26 m height, 0.80 m diameter, 130 L volume) composed of nylon mesh (3 mm) and a polypropylene baffle (Figure 3.1.1). The caged snails were exposed in situ at two sites (site A and site B), representing different pollution levels of the Taihu lake (Figure 3.1.2). At each site, three cages, containing 120 snails each, were immersed into the water and fixed in direct contact with the sediments. One cage from each site was sampled each after 7, 14 and 21 days, respectively. However, one cage located at site A disappeared for unknown reasons after 21 day of exposure, and thus it was impossible to recover the snails from this cage. At each of these time points, the battery of biomarkers were used in the study (c.f. above).

Analyses of selected contaminants in sediments of Taihu Lake showed that metals (Cr, Cu, Pb, Ni, Zn, Cd, As), organochlorine pesticides (OCPs), PCBs and the flame retardants, the PBDEs, were detectable at both caging sites. Concentrations of trace elements (Cu, Ni and As) and the DDT metabolite, 4,4'-DDE, exceeded their corresponding threshold effect level (Tel) according to the sediment quality assessment values for freshwater ecosystems if Canadian regulations are applied, indicating that adverse biological effects may occur (Figure 3.1.3). All concentrations of chemicals analyzed in the sediment collected from site A, except for PBDEs, were greater than those at site B. This is in accordance with the result that the survival rate of *B. aeruginosa* caged at site A was lower than that of snails caged at site B.
after different exposure times (7 and 14 days), indicating that it was a relatively sensitive species for biomonitoring in Taihu Lake.

With regard to the biochemical analysis (Figure 3.1.4.), EROD activities in hepatopancreas tissues of snails were significantly induced at the beginning of exposure period (7 days) and then tended to have no significant difference with that of the control with the increasing exposure time, which indicated an adaptation of the caged snails for the contaminated sediments at both sites in Taihu Lake. During the exposure, significant increase in oxidative stress as measured by ROS concentrations as well as LPO and PCC occurred in snails exposed at both sampling locations. In addition, significant increases in SOD and CAT activities were observed, which suggested an adaptive response of the snails inhabiting contaminated sites in Taihu Lake.

The Integrated Biomarker Response Index (IBR) can form discriminating scores to describe toxically induced stress based on the specific responses of a number of biomarkers across different sampling sites. In this study, it was used as a tool for the visualization of biological effects of contaminants on snails caged at both sites in Taihu Lake. In general, the IBR index (stress index) based on the biomarkers of EROD, ROS, SOD, CAT, LPO and PCC in the hepatopancreas tissue of B. aeruginosa increased from day 7 to day 14 of the exposure and decreased at day 21, showing that environmental stress effects in B. aeruginosa initially increased and then weakened, which might be an adaptation mechanism for B. aeruginosa to toxic effects of environmental contaminants. The IBR values were higher in the snails from site A on the 14th day of exposure compared to site B, indicating that contaminants at site A had a greater impact on B. aeruginosa. As higher contamination levels of metals, OCPs and PCBs were found in the sediments collected from site A compared to site B, the IBR index proved to agree with the levels of environmental contamination in different sediments from Taihu Lake. Thus, it could
Figure 3.1.4. Biochemical parameters in the hepatopancreas of snails (*Bellamya aeruginosa*) caged at sites A and B during the exposure in Taihu Lake.
be inferred that the integration of multiple biomarker responses found in caged snails could reflect the contamination levels measured at different sites and used as an efficient biomonitoring approach.

In conclusion, the biochemical responses in caged snails deployed at two sites in Taihu Lake as well as the chemical analysis of selected organic and inorganic pollutants indicated significant anthropogenic pollution and potential risks to resident organisms. Multiple biomarkers including EROD, ROS, SOD, CAT, LPO and PCC were induced during the caging exposure experiment. The IBR results showed that the CAT activity was the most sensitive biomarker. The results of the biochemical biomarkers in B. aeruginosa were consistent with the chemical contamination in the sediments of the lake, indicating that in situ exposures with caged snails can serve as an efficient biomonitoring approach to evaluate sediment quality.

**Key message/Implications**
- The pond snail B. aeruginosa is a relatively sensitive species in the biomarker test battery implying the applicability for biomonitoring in Taihu Lake and elsewhere.
- In situ exposure of caged snails can serve as an efficient biomonitoring approach to evaluate sediment quality.

**Publications from the subproject**

**Researchers involved in the project**
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Magnus Breitholtz, Ling Chen, Markus Hecker, Taowu Ma, Yanling Qiu, Jianfu Zhao, Lei Duan, Qian Li, Meng Wang


3.2 Ecotoxicology of chlorinated paraffins

**Objectives**
Water quality is of critical importance, for humans and for wildlife. Accordingly the environmental occurrence and toxicity of pollutants are keys to understand and manage chemical pollution in the aquatic ecosystems and for human health. This project has been working on bioaccumulation and aquatic ecotoxicity to generate new knowledge on Chlorinated Paraffins (CPs), which are needed for development of adequate regulations for these high volume and technically challenging chemicals. Therefore, our objectives were:

- to establish a passive dosing test system with silicone to ensure stable exposure concentrations in water.
- to determine partitioning coefficients between water, *Daphnia magna* and silicone.
- to calculate BCF and BAF values in *Daphnia*.
- assess effects of CP exposure on life history traits and lipid profile in *D. magna*.

**Main results**
Aquatic toxicity testing is a part of the environmental risk assessment of industrial chemicals, such as CPs, and several OECD guidelines have been developed for this purpose. Environmental risk assessment is traditionally based on chemical testing with aquatic organisms through water exposure via solvents (e.g., spiking chemicals in water solubilized in DMSO, methanol or acetone). However, CPs are practically insoluble in water and most organic solvents, which complicates their aquatic toxicity testing and hampers environmental risk assessment. To overcome these methodological problems, we need alternative testing methods.

Passive dosing is a technique that employs a silicone polymer dosed with hydrophobic chemicals as an alternative to a solvent carrier to achieve stable concentrations in water. In this project, the first step was to validate passive dosing for the aquatic toxicity testing of CPs. Using different commercial CPs representing short- (SC-), medium- (MC-) and long-chain (LC-)CPs, we showed that a stable exposure in water is possible after 24 hours of silicone-water contact lasting for up to one week, i.e.,
a period sufficient for toxicity testing. This approach allowed us to better understand the behavior of CPs in water: carbon chain length is expected to be the most important property affecting the water solubility of these substances, and so SCCPs are expected to be more soluble in water than MCCPs and LCCPs. However, in this work, we observed that not only the carbon chain length but also the chlorine content contributes significantly to the overall solubility of CP substances in water, so that MCCPs with low chlorine content can be as hydrophobic as SCCPs with high chlorine content. Since these chemicals vary in both carbon chain length and chlorine content, the assessment of thermodynamic
properties, such as water solubility, vapor pressure, and boiling point, in CPs is complicated. Each category of CPs will assume a range of values for each property, overlapping between the categories, rather than a single value. Additionally, a commercial CP substance can be composed of several hundreds of CP congeners and thousands of isomers that will individually achieve equilibrium concentrations in water, that can add up to significant water concentrations (mg L\textsuperscript{-1} range). These aspects highlight the inherent complexity of this group of chemicals, which has led CPs to be added to the UVCBs (substances of Unknown or Variable composition, Complex reaction products, or Biological materials) group of chemicals, under European regulation.

Once a validated exposure system was developed, a standard test organism in ecotoxicology, the crustacean *Daphnia magna* was used to understand if silicone-dosed CPs in water were taken up by the animals introduced to the passive dosing system. This allowed us to estimate organism-water partitioning coefficients for different CP commercial substances from stable water exposures; this was a novel contribution to the field. Given the high water to organisms partitioning of CPs, our findings indicated a bioaccumulation potential for these substances.

Under the multilateral agreement for the protection of the environmental and human health, the Stockholm Convention, organic chemicals widely found in the environment should be assessed for Persistence (P) in the environment, Bioaccumulation (B) in aquatic and terrestrial biota, and Toxicity (T) towards humans and wildlife. When chemicals are demonstrated to meet these criteria, they are labelled as Persistent Organic Pollutants (POPs) and thereafter regulatory action is advised to follow by the state parties. In 2016, the Stockholm Convention added SCCPs to the POPs list based on their PBT properties, and advised towards the elimination of these chemicals. MCCPs and LCCPs were listed as alternative products by the Stockholm Convention. Therefore, it was of interest to assess the Bioaccumulative (B) and Toxic (T) potential of the substitution products and compare to the restricted chemicals.

To assess the bioaccumulative potential of CPs in aquatic biota, we conducted laboratory bioconcentration (water-organism transfer) and bioaccumulation (both water- and diet-organism transfer) experiments with *D. magna* using passive dosing. We observed that all CP substances (SC, MC and LCCPs) were bioaccumulative in *D. magna*, with bioaccumulation factors between 6.5–7.0 (log BAF, L kg lipid\textsuperscript{-1}). Concerning bioaccumulation factors, the chlorine content and carbon chain length acted concomitantly in increasing the hydrophobicity of the substances and therefore bioaccumulation factors. We also found that *D. magna* depurated the CP commercial mixtures relatively fast, with half-lives of two to ten hours; therefore, metabolism can be a relevant elimination pathway for low chlorinated CPs.

Additionally, we showed that LCCPs, the substitution products of SCCPs already being found in the environment, have the potential to bioaccumulate in aquatic biota. LCCPs are considered the most hydrophobic group of CPs, and represent the group with the highest molecular weight chemicals. Such chemicals are usually not considered during environmental risk assessment since they are not expected to be found in biota. However, in this study, we showed that, during stable water exposure, poorly soluble and high molecular weight chemicals can be taken up and accumulated in *D. magna*, raising concerns towards commonly applied cutoffs during the risk assessment of chemicals.
Finally, the passive dosing system was used to assess the CP exposure effects on the reproductive output of *D. magna*. Reproduction effects in *D. magna* are included in the OECD guideline battery (Test No. 211: *Daphnia magna* Reproduction Test), and commonly used for the risk assessment of chemicals. In this test, newborn female *Daphnia* are exposed to the test substance for 21 days. During this period, life-history traits such as survival and reproductive output are recorded. We found that the reproduction output of *D. magna* was negatively affected by the chronic exposure to two CP substances: a SCCP with 70% chlorine content, and a CP with 52% chlorine content composed of SC, MC and LCCPs. Moreover, the fatty acid composition was significantly altered in the exposed animals.

In this subproject we advanced the knowledge on the B and T assessment of the short, medium and long chain CPs, which is valuable for the on-going policy discussion and possible regulatory action towards these two groups. Considering the growing environmental detection in different matrices and growing production of MCCP and LCCPs, there is still a need for more data mainly regarding the Persistency assessment and of further monitoring studies in remote and non-remote regions to understand spatial and temporal trends.

**Key message/Implications**

– Carbon chain length and chlorine content can be equally important in the environmental fate of CPs, and therefore the bioaccumulative potential of in-use MCCPs and LCCPs may be equal or higher than the currently restricted SCCPs.

– CPs bioaccumulate in aquatic organisms reaching toxic concentrations; they are detrimental for survival and reproductive output. They also induce alterations in the lipid composition of the test organisms during the chronic exposure.

**Publications from the subproject**


**Researchers involved in the project**

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3.3 Establishment of *Bellamya aeruginosa* as a research monitoring species in the Yangtze River Delta

**Objectives**
Strategies for sampling is of critical importance for all monitoring being planned and done independent of if it is in humans or wildlife. It is a matter of quality of knowledge to pursue this in the most competent way. The aims of this project have been:
- to establish a scientifically based structure for sampling and storing of snails for the Yangtze ESB (Environmental Specimen Bank) and to design a future monitoring program of selected POPs in the Yangtze River Delta (YRD).
- to design the program by considering statistical power. Several sampling strategies were compared in combination with chemical analysis and computer simulations.
- to analyze and report temporal trends and geographical differences of persistent and bioaccumulative organic contaminants from snail samples, collected during the period.
- to expand the experience to Sweden by introducing another species of snail (*Viviparus viviparus*) in Swedish lakes, as a new monitoring species.

**Main results**
Environmental biomonitoring includes processes and activities to assess the quality of the environment by using biological samples as indicators. A number of wildlife species, covering a wide range of trophic levels, including mussel, fish, seal and bird egg, have been used in Swedish biomonitoring programs since the 1960’s. The design of environmental biomonitoring programs is highly important. The programs must enable us to observe changes in the burden of environmental pollutants or biomarker responses and given that the budget is a limiting factor, it is of utmost importance to use proper species and sampling strategies.
In the Chemstrres project, the pond snail (*Bellamya aeruginosa*) was used as a monitoring species. The pond snail was also used to illustrate how different sampling strategies may influence the monitoring results for e.g. temporal trend assessments. Further, spatial distribution and temporal trends of selected POPs in the YRD environment was demonstrated by using the pond snail as an indicator species.

Historically, YRD is a famous area for agriculture and aquaculture in China. Lower trophic level organisms are often selected to assess the contamination situation of pollutants in local areas as well as to evaluate the risk of top-predators being exposed to environmental contaminants. Their relatively small home ranges make them representative of well-defined areas. Their low metabolic capacity makes them suitable as indicators for substances degraded by species at higher trophic levels with a potentially higher metabolic capacity (e.g. polycyclic aromatic hydrocarbons (PAHs)).

The pond snail is an abundant aquatic organism in the YRD and is also used for human consumption. There are a few studies showing the environmental contamination situation of pollutants in local areas as well as to evaluate the risk of top-predators being exposed to environmental contaminants. Their relatively small home ranges make them representative of well-defined areas. Their low metabolic capacity makes them suitable as indicators for substances degraded by species at higher trophic levels with a potentially higher metabolic capacity (e.g. polycyclic aromatic hydrocarbons (PAHs)).

**Figure 3.3.1a** (left). Box-plots (diamond = 50% of population, whiskers = 1.5 x IQR (Interquartile Range)) example of median PBDEs, orange diamonds = China, blue = Sweden.  
**Figure 3.3.1b** (right). Multiple regression analysis showing the influence of some potential confounding factors on, the flame retardant, BDE-153 concentration in the pond snail, LW=Lipid weight increasing concentrations with increasing lipid weight (p<0.001), WSB=Whole Soft Body weight, decreasing concentrations with increasing weight (p<0.05), length, no significant correlation (note that only 38 % of the variation in concentration can be explained by LW, WSB and length, the rest is measurement errors and factors not included in the analysis).
exposure and bioaccumulation of contaminants in snails in aquatic ecosystems (Kobayashi et al. 2015, She et al. 2013). The pond snail has also been selected for sediment toxicity testing (Ma, et al. 2010, see also Chapter 3.1 above).

A number of factors need to be carefully considered when a new monitoring program is to be establish. Choosing tissue type is important e.g. POPs tend to accumulate in lipids, whereas phenolic compounds tend to accumulate in blood. For snail samples, the whole soft tissue is the only practical option for chemical analysis. Another important issue is whether to use pooled samples or individual samples for analyses. In general, individual analysis is preferred when the budget is sufficiently large. Advantages of individual analyses are e.g. to assess relationships between the studied contaminant and potential confounding factors like age (size may sometimes be used as a proxy for age) and lipid content (Figure 3.3.1a). It is also important to describe the concentration distribution, e.g. to find the 90th percentile that can be used in risk assessments. By recording data on confounding factors at collection and preparing samples for chemical analyses (Figure 3.3.1b), concentrations can be adjusted accordingly and the variation reduced (that implies increased power). A combination of individual samples and pooled samples may prove cost-efficient. When a new site is established, individual samples can be used to get a picture of the individual variation and to correlate potential confounding variables with concentrations (Figure 3.3.1b).

A future monitoring program can use pooled samples for chemical analyses within an ongoing program, but keep individual specimens stored in the specimen bank for future retrospective special studies where individual samples can be used. Given that sufficient knowledge on properties of pollutants and species is available, pooling might be the best choice (Bignert et al. 2014). Pooling can also save cost for chemical analyses that can be used to expand the number of sampling sites and increase the geographical coverage. Pooling may also be necessary if individual samples cannot provide enough sample material for chemical analysis, and snails have in general a low fat content which might increase the amount of material needed for chemical analysis.

Worryingly, many international monitoring programs have a too poor statistical power to detect the changes that one expects to be able to detect (Bignert et al., 2004). To ensure that results from monitoring programs can be used to detect temporal trend and geographical differences with any confidence, it is important to consider Type I (α) and Type II (β) errors, when analyzing the results statistically. Statistical power (1 - β) is defined as the probability to detect a trend/difference, if it actually exists. To estimate the statistical power is essential in designing and evaluating monitoring programs. The Type I error is set, prior to any statistical analyses, at a level more or less commonly agreed upon (usually α = 0.05), and is the risk taken to reject the null hypothesis (i.e. no change, no difference) when it is actually false. The Type II error is the risk to ignore a true trend or difference. The Type II-error depends on several factors, the sample size, number of samples per year; within- and between-year variation; the Type I error selected; effect size; and characteristics of the statistical test being applied (e.g. Gewurtz 2011, Ellis 2010, Bignert et al. 2004; Fryer and Nicholson 1993; Sokal and Rohlf 1995). To avoid poor statistical power in monitoring programs, the required effect size (i.e. how big is the trend/difference that needs to be detected, should it occur) and the Type-II error should be defined before the sampling program is determined to ensure that sufficient samples sizes can be afforded within the given budget.
Chapter 3: Chemstrres research: Main results and messages

It is important that the various projects within Chemstrres are integrated as much as possible, e.g., the fish project to explore the biomagnification in the food web, and snail eco-toxicology to strengthen snail as a monitoring species in the YRD.

Snails and sediments were collected from three lakes (Tianmu Lake, Taihu Lake and Dianshan Lake) in YRD in 2014. No consistent significant correlations for PBDEs were found between snails and sediments. Initially, Tianmu Lake was chosen as a reference site as it is located far from urban areas. However, chlorinated paraffins (CPs) were detected in the pond snails, indicating an influence from anthropogenic sources. Still, the sampling activities continue but currently with a focus on Taihu Lake area. The sampling of pond snails in the YRD also encouraged the sampling activities in Sweden of the snail species *Viviparus viviparus* to serve as a potential freshwater indicator species, especially for PAHs but also for other contaminants.

Chemical analyses were performed on individual snail from Tianmu Lake, China and Lake Tärnan, Sweden. For both lakes, each of ten individual snails from five sites were analyzed. The chemical analyses showed a general low level of the industrial POPs, e.g. PBDEs and PCBs. Lake Tärnan is considered as a reference lake under the Swedish monitoring program.

High concentrations of DDE and pentachloroanisole were detected in the Tianmu Lake pond snails. DDE is a metabolite of DDT, whereas pentachloroanisole is a methylated form of pentachlorophenol (PCP). The occurrences of these chemicals indicate previous or present use in the area.

Concentration of the flame retardant compound, BDE-47, in Tianmu Lake snails were selected for computer simulation. The within- and between-site variance of BDE-47 in the snails represents other polybrominated diphenyl ether (PBDE) congeners quite well. Computer simulation of various sampling strategies, showed that "convenient" sampling (i.e. going to the closest spot from the road, collecting all the specimens at the same spot) should be avoided, and sampling should be spread over a wide range area, to promote best quality monitoring of temporal trends of pollutants see Yin et al. (2017) for further details.

**Key message/Implications**

– Invertebrates like snails may serve well as indicator species for contaminants especially for e.g. PAHs that is more likely to be metabolized in vertebrate species. The pond snail is particularly interesting since it is used for eco-toxicological studies and as human food.

– Considering statistical power along with logistics when defining the sampling strategy for monitoring activities and specimen banking will improve quality and avoid waste of money.

– Computer aided simulation of various sampling strategies, using data on within- and between-site variation from pilot field studies, to achieve realistic statistical power estimates, can save money.

**Publications from the subproject**


Ge Yin, Yihui Zhou*, Anna Strid, Ziye Zheng, Anders Bignert, Taowu Ma, Ioannis Athanassiadis, Yanling Qiu. Spatial distribution and bioaccumulation of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in snails (Bellamya aeruginosa) and sediments from Taihu Lake area, China. Environmental Science and Pollution Research. 2017,24(8):7740-7751.

Researchers involved in the project
Profs. Yanling Qiu (Email: ylqiu@tongji.edu.cn), Anders Bignert, Taowu Ma, Åke Bergman; Ms. Sara Danielsson, Drs. Ge Yin, Yihui Zhou, Elisabeth Nyberg and Mr. Ioannis Athanassiadis

References
Chapter 3: Chemstrres research: Main results and messages

3.4 Drinking water contaminants – a screening project

Objectives
Clean drinking water is a prerequisite for good health and wellbeing. Our environment, cities need to be sustainable as well as our water sources.

The present subproject of Chemstrres has addressed the following objectives:
– to establish a research monitoring framework for human exposure to drinking water.
– to screen legacy pollutants, endocrine disruptors, and other emerging contaminants in drinking water.
– to identify and quantify some typical contaminants including plasticizers (e.g. phthalates), thermal stabilizers (e.g. organotin compounds), flame retardants (e.g. organophosphates) and fluorosurfactants (e.g. PFOS and PFOA) in drinking water, especially in freshly purified water from Automatic Vending Water Machines.

The project workflow is visualized in Figure 3.4.1.

Main results

Preliminary screening of emerging contaminants in drinking water from water-producing machines in public places.

In the current Standards for Drinking Water Quality in China and other countries, regulatory indicators include some organic pollutants such as pesticides, disinfection by-products and benzene series. However, endocrine disruptors and emerging contaminants are continuously detected in drinking water, but few of them are included in the scope of water quality supervision in many countries. So what are the characteristic contaminants in urban drinking water? For this purpose, this project first used solid phase extraction and comprehensive two-dimensional GCxGC-MS to conduct non-target screening of organic contaminants for drinking water in public places in Shanghai. The results showed that polycyclic aromatic hydrocarbons (PAHs), phthalate esters (PAEs), triazine pesticides, phenols and bisphenols, pharmaceutical
Figure 3.4.1. Illustration of the subproject workflow.

Figure 3.4.2. Two-dimensional gas chromatograms as fingerprints of organic contaminants in drinking water from Shanghai. (a) Freshly purified water from water vending machine; (b) Hot water from water boiling machine.
active compounds, organophosphorus flame retardants (OPFRs) and some polar compounds were generally detected as shown in Figure 3.4.2. Many of them are related to plastic additives, which have endocrine disruption or carcinogenic effects and may become classified as emerging contaminants in drinking water.

**Occurrence of plastic-related chemicals in drinking water in Shanghai**
Contamination of phthalates, low substituted organotin, organophosphates, per- and polyfluoroalkyl substances (PFASs) commonly occurs in drinking water in Shanghai. Many of them are
widely used as plasticizers, heat stabilizers, flame retardants, hydrophobic and oil dispersing agents for plastic products. The concentrations of these contaminants were quite different in various samples of drinking water. The overall contamination level of drinking water was lower than the regulation limits in the relevant standards of China, the European Union and the United States. Consequently, this indicate a low risk of human exposure to plastic-related chemicals through drinking water, alone. It is important to consider also other exposure sources, as well.

Some toxic substances, less frequently reported in the past, were also detected in drinking water in Shanghai. Dihexyl phthalate, which is used as plasticizer for PVC, rubbers and cellulose esters and was shown to activate Nrf-2-mediated antioxidant response in human cell line, mainly exists together with dibutyl phthalate (DBP) and diisobutyl phthalate (DIBP) in bottled/barreled water in this study. Attention should be paid to the sources of this substance in drinking water products (e.g. bottled water) and its impact on human body, and related monitoring and research need to be strengthened.

The presence of plastic-related contaminants in commercial drinking water, including freshly purified water and bottled (barreled) water, is lower than that of tap water and centralized supplied hot water as shown in Figure 3.4.3. As far as PAEs, organotin, OPFRs, PFASs are concerned, they can be effectively removed after advanced treatment, hence improving the drinking water quality. Conventional water treatment in waterworks, traditional heating treatment (boiling water) and improperly maintained water vending machines cannot effectively remove PAEs, organotin and PFASs in water. The concentrations of highly polar PFASs in tap water after conventional water treatment were even higher than those in raw water, while most of plastic-related contaminants can be effectively removed by advanced treatment.

In the past twenty years, the drinking habits of urban residents have gradually shifted from tap water to commercialized purified water products. The improvement of consumption habits can obviously reduce the daily intake of various organic contaminants. Therefore, drinking water safety can be enhanced by increased portable water consumption and improved water processing technology.

**Key message/Implications**
- A large number of contaminants are present in various drinking water in Shanghai, and some highly polar pollutants are not effectively reduced after conventional treatment
- The advanced water treatment of water vending machines can effectively remove trace organic contaminants in tap water and improve the water quality, but the equipment needs to be properly maintained and managed.
- To enhance the safety of drinking water, it is necessary to encourage consumption of purified water products and improve water treatment technology.

**Publications from the subproject**
and their removal from two urban drinking water treatment plants and a constructed wetland. *Environmental Science and Pollution Research* 24; 14889–14902 url: https://doi.org/10.1007/s11356-017-8830-y (Open Access).


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3.5 Sewage sludge as a mirror of human activities

Objectives
Sewage sludge is a mirror of our modern life in the cities, the buildings and constructions and from the infrastructures, transportation, what is being produced and consumed. The chemical emissions reach the sewage systems and end up to a large proportion in the wastewater treatment plants. This project has accordingly been designed to meet the aims:
– to create a database of chemicals present in sludge, that would assist engineers, researchers, and policymakers in developing a disposal technology necessary for effectively addressing the occurrence, fate, and the level of risk of contaminants being present in sludge-applied soil.
– to design an optimal sampling strategy for developing a Chinese National Sewage Sludge Survey (CNSSS), the main contents of this study are:
  • to examine the distribution of WWTPs by urban agglomeration, wastewater treatment process, and wastewater treatment capacity
  • to propose sampling strategy based on statistical theory and Monte Carlo simulation.

Main results
A broad spectrum of chemicals currently being produced and consumed are often unintentionally washed down the drain and entered into municipal wastewater treatment plants (WWTPs) (Meng et al. 2017). As a by-product which is produced during wastewater treatment, sewage sludge may contain a variety of chemicals when discharged. When sludge is disposed illegally and/or applied as soil fertilizer, these chemicals will be released into the environment as contaminants, posing risks to both the environment and human health. On the other hand, sludge has been used as a matrix to explore the fluxes, temporal trends, and sources of chemicals discharged by human society. Therefore, the development of a regional or nationwide database of chemicals in sludge is a matter of urgency, that would assist engineers, researchers, and policymakers in developing a disposal technology necessary for effectively addressing and understanding the issue of sludge, its occurrence, fate, as well as the level of risk in respect of contaminants being present in sludge-applied soil.
Four nationwide sludge surveys were conducted in 1982, 1988–1989, 2001, and 2006–2007 by the United States Environmental Protection Agency (U.S. EPA 2009), which were aimed at identifying and quantifying priority contaminants in sludge. In 2011, the European Commission's Joint Research Centre screened 114 analytes in 61 sludge samples collected from 15 European countries (JRC 2012). Similar investigations have been conducted in the United Kingdom, Spain, Germany, Sweden, Switzerland, and Australia. In a review from China, studies have shown 35 classes of organic chemicals consisting of 749 individual compounds and one mixture to be present in Chinese sludge since 1987 (Meng et al. 2016).

The abovementioned surveys provide a plethora of information on chemicals in sludge. However, the majority of these studies (except the fourth survey in the U.S.) only gave cursory information on the number and locations of WWTPs sampled, and their sampling work is arbitrary and possess a lack of statistical rigor, which may result in the concentrations and compositions of contaminants assessed, being biased. For example, Yang, et al. (2014) sampled sewage sludge from 107 WWTPs in 48 cities covering 31 provinces, autonomous regions, and municipalities, as well as Hong Kong, Macao, and Taiwan, without essential sampling details illustrating sample representativeness or sampling error. Representative sampling was introduced already by Anders Kiaer (Thomsen, 2001; Jin et al. 2015). It proved that random samples could mirror the characteristics of the studied population. Such random sampling includes simple random sampling, stratified sampling, systematic sampling, cluster sampling, and multistage sampling. Among them, stratified sampling could ensure that the allocation of the sample is similar to that of the sampled population of measurements, and hence effectively improve the precision of the estimates. Moreover, stratified sampling was successfully employed in the fourth sewage sludge survey in the United States (U.S. EPA 2009).

In total, China has 3600 WWTPs. The Yangtze Delta Urban Agglomeration (around Shanghai) has the highest number of WWTPs (614), followed by the Beijing-Tianjin-Hebei Urban Agglomeration (around Beijing and Tianjin; 306), the Middle Yangtze River Urban Agglomeration (267), the Shandong Peninsula Urban Agglomeration (266), and the Pearl River Delta Urban Agglomeration (around Guangzhou; 224). Ten types of treatment processes were employed in China's WWTPs, including oxidation ditch with a percentage of 28.7%, anaerobic-anoxic-oxic (28.1%), cyclic activated sludge system (12.8%), anaerobic-oxic (7.3%), sequencing batch reactor (6.9%), biological film (4.4%), traditional activated sludge (3.3%), biological lake (2.9%), and other treatment process (5.6%). For treatment capacity, the investigated WWTPs were divided into six groups based on daily treatment flow (t/day), i.e., super-small capacity (1400 ≤ F < 5000), small capacity (5000 ≤ F < 20,000), medium capacity (20,000 ≤ F < 100,000), large capacity (100,000 ≤ F < 500,000), super-large capacity (500,000 ≤ F < 2,800,000), and others.

How to choose WWTPs in the Chinese National Sewage Sludge Survey? First, we confirm the sampling frame, consisting of 3349 WWTPs among the 3600 WWTPs. Second, we determine the target indictor of the survey dry sludge production for testing the sampling strategy. Dry sludge production was calculated from wastewater treatment flow and can be influenced by urban agglomeration, treatment process, and treatment capacity. Urban agglomeration is a comprehensive geographic indicator, representing the production, consumption, and discharge of chemicals in a specific area. Wastewater treatment process is an indicator of all technologies used to
remove chemicals during wastewater treatment whilst wastewater treatment capacity is an indicator related to the mass/concentrations of chemicals in influent and/or effluent, as well as sludge. Therefore, urban agglomeration, wastewater treatment process, and wastewater treatment capacity were employed as stratification indicators.

For each sampling strategy, a Monte Carlo simulation was performed 10,000 times to examine the sampling campaign by MATLAB (R2018a; Mathworks). Then, the mean dry sludge production of the selected WWTPs in each stratum was adopted to extrapolate the total dry sludge production of the total 3349 WWTPs. The 10,000 estimated total dry sludge production were used to calculate root mean square error (RMSE), together with the true total dry sludge production of 3349 WWTPs (6.06 million tonnes/year). Figure 3.5.1 shows the Gauss fitting curves (all $R^2 > 0.97$) for the frequency distribution of the 10,000 estimated total dry sludge productions for each strategy. Clearly, Strategy IV, stratified random sampling strategy based on treatment capacity, presents the smallest RMSE among all strategies and provides higher precision (i.e., variation among all the estimated data) and lower bias (i.e., difference between the mean of all estimated total dry production and the true total production).

By multiple stratifications, Strategy VI, Strategy VII, and Strategy VIII get more precise results. In particular, Strategy VIII with 480 strata, by the use of three stratification indicators, reaches the highest precision in all strategies. However, excessive stratification leads to large bias, which will underestimate the total dry sludge production. In light of bias, urban agglomeration was the worst stratification indicator whilst treatment process was slightly better, and treatment capacity was the best. Any combination of these stratification indicators will increase the bias. Thus Strategy VIII (the combination of urban agglomeration, treatment process, and treatment capacity) is the worst, followed by Strategy Strategy VI (the combination of
urban agglomeration and treatment capacity), and Strategy VII (the combination of treatment process and treatment capacity), in descending order of bias. Several limitations in available official data bases have been identified by this study. One such limitation includes the lack of information available in respect of WWTPs. WWTP lists are issued by various government departments and are considerably different from each other and sometimes incomplete e.g. lack of information on wastewater sources, and thus difficult to compare or use in overall assessment. A general platform from which interested parties or researchers may collect WWTP information does not exist. Researchers are therefore referred to collecting information related to WWTPs from multiple sources. This, once again, increases the risk of incomplete information being obtained.

In respect to stratification indicators, there are two identifiable limitations to the study. First, the stratification indicators used in this study are limited due to the lack of information provided by the relevant government departments and available resources. Second, the classification of stratification indicators requires further consideration. In respect of the sampling model, there are also two notable limitations. The first is that the focus of this study is singular in nature, with its focus being on decreasing sampling error.

Sampling cost is also an essential factor which should be considered. As such, it is suggested that models involving cost should be introduced to future sampling strategy designs. The second is that this study only applies simple random sampling to each stratum. Other sampling methods such as systematic sampling can also be employed for sampling within strata in subsequent studies.

Key message/Implications
- The distribution of WWTPs by urban agglomeration, wastewater treatment process, and wastewater treatment capacity has been examined.
- Statistical sampling was developed for Chinese national sewage sludge survey. Stratified random sampling based on treatment capacity proved to provide the optimal representation. While excessive stratification may increase bias.

Scientific Publications from the subproject

Researchers involved in the project
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References

3.6 Assessing persistent and bioaccumulative compounds in wildlife from the Yangtze River Delta

Objectives
Persistent organic pollutants (POPs) and chemicals with persistent and bioaccumulative properties are of global concern since they find their way to the environment via leakage and discharges during production and through their mobility, leaking out of materials and goods. POPs are contaminating land and waters, the life there and ultimately our drinking water and food, threatening both human health and the wellbeing of wildlife.

The aims of this project have been:
- to screen for persistent bioaccumulative compounds in wildlife from the YRD.
- to determine concentration and congener profiles of persistent and bioaccumulating environmental pollutants in wildlife species.
- to search for, and potentially identify sources of indicated pollutants.
- to select species for research monitoring of persistent and bioaccumulative environmental pollutants in wildlife.

Main results
It is well established that observations from the environment, i.e. wildlife, has been instrumental for identification of new environmental pollutants and also health effects and disorder among wildlife, such as birds of prey, high trophic mammals and snails (IPCS, 2002; UNEP/WHO, 2013). The present work has been closely related to work done in Sweden during the last half decade and to the advanced environmental monitoring in Sweden. The establishment of the Yangtze Environmental Specimen Bank in Jiaxing has been instrumental for the present project collecting and storing wildlife samples for chemical analyses, screening and monitoring.

To meet the aims of the present project, research focused on screening for pollutants in wildlife from the YRD. In a first published study (Zhou et al. 2016a), samples of rice field eel (Monopterus albus, RFE), asiatic toad (Bufo gargarizans, AT), dark-spotted frog (Pelophylax nigromaculatus, DSF), short-tailed mamushi snake (Gloydius brevicaudus,
STM), chinese pond-heron (*Ardeola bacchus*, CPH) and peregrine falcon (*Falco peregrinus*, PF) were analysed for persistent and bioaccumulative halogenated substances. The occurrence of pollutants is exemplified in Figure 3.6.1. for two of the species (STM and PF). As shown, these wildlife species are entirely dominated by chlorinated paraffins (CPs) reported in very high concentrations (ppm levels) (Zhou *et al.* 2016a). Levels of the POPs analyzed and listed are only making up a small proportion of the total pollutant burden in the wildlife studied as visualized for the two species (Figure 3.6.1.). The first study has been followed up by a second study on an additional number of wildlife from the Yangtze river delta area (Du *et al.* 2018) addressing specifically the CP contamination. Also the CP concentrations were in the ppm range but somewhat lower than in the first report. The latter study reports the CPs divided into the three groups of short, medium and long chain CPs (SCCP, MCCP and LCCP, respectively). The relative abundance of these CP classes is visualized for their occurrence in peregrine falcon and short-tailed mamushi snake in Figure 3.6.2.
The results on CPs from our studies are in line with other reports of pollutants in wildlife from China and may be compared to data from Arctic wildlife as recently reviewed by Vorkamp et al. 2019.

A freshwater food web from Dianshan Lake were also investigated for SCCPs (Zhou et al. 2018). Fourteen species of aquatic organisms, fish, clams, snails and shrimps were analysed for CPs and high concentrations of SCCPs were found (Figure 3.6.3.). The highest average concentration, on a fat weight basis, was found in common carp (Cyprinus carpio), followed by the pond snail (Bellamya aeruginosa), common carp (crucian carp (Carassius auratus) and predatory carp (Chanodichthys erythropterus). The concentrations for all of these four species are above 150 and below 300 µg g-1 fat. Average concentrations and range are shown in Figure 3.6.3 for the four species as well as for the other species studied herein. It is notable that this is only SCCP and the question is how much of MCCP and LCCP that these fish species, the pond snail and other invertebrates may have accumulated. A chain length of C10 and C11 and chlorine content of Cl 6-7 are most common, particularly in the benthic organisms (Zhou et al. 2018). The subproject 3.9 is further elaborating on fish for monitoring.

The data obtained within Chemstrres clearly show the POP characteristics of the CPs are independent of their chain lengths. Accordingly, it is time to also include MCCPs and LCCPs in the Stockholm Convention.

The screening of the wildlife as presented by Zhou et al 2016a included several of the well-known POPs. The log scale concentrations of β-hexachlorocyclohexane (β-HCH), DDT, DDE, Mirex, HCB, the polychlorinated biphenyl, CB-118 and the brominated flame retardant BDE-153, a hexabrominated diphenyl ether are presented in a diagram (Figure 3.6.4) for the six species specified above plus whiskered tern (Chlidonias hybrida) and black-crowned night heron (Nycticorax nycticorax).
Figure 3.6.3. Concentrations of short chain chlorinated paraffins in fish and invertebrates from Dianshan Lake (Zhou et al. 2018). The species analyzed were among the carp fish: grass carp (GRC), bighead carp (BHC), silver carp (SVC), crucian carp (CCC), common carp (CMC), predatory carp (PDC); among other fish species: snakehead (SNH), yellow catfish (YCF), rosy bitterling (RBL), stone moroko (STM), bigmouth grenadier anchovy (BGA) and among invertebrates: clam (CLM), pond snail (SNL) and shrimp (SHP).

Figure 3.6.4. Concentrations (ng g⁻¹ fat) of β-hexachlorocyclohexane (β-HCH), DDT, DDE, Mirex, hexachlorobenzene (HCB), the polychlorinated biphenyl, CB-118 and the brominated flame retardant BDE-153 in rice field eel (Monopterus albus, RFE), asiatic toad (Bufo gargarizans, AT), dark-spotted frog (Pelophylax nigromaculatus, DSF), short-tailed mamushi snake (Gloydius brevicaudus, STM), whiskered tern (Chlidonias hybrida, WT), chinese pond-heron (Ardeola bacchus, CPH), black-crowned night-heron (Nycticorax nycticorax, BCNH) and peregrine falcon (Falco peregrinus, PF).

Among the POPs, DDE is accumulated at the highest concentrations in seven out of the eight wildlife species analysed. The DDE concentrations are up in the ppm (µg g⁻¹ fat) range. This is indicative of very recent or even ongoing use of DDT as a pesticide. Another pesticide, i.e. β-HCH, is in these wildlife species at only slightly lower concentration than DDE. The PCBs, represented in Figure 3.6.3 by CB-118, is significantly lower than the DDE and even lower than the CB-118, is the brominated flame retardant, BDE-153. The ubiquitous global pollutant, HCB, varies a lot between the species analysed (cf. Figure 3.6.4).

A novel PCBs contamination pattern was detected, a pattern characterised by highly chlorinated biphenyls (PCBs with eight to ten chlorines), in eggs from the black-crowned night heron and whiskered tern (Zhou et al 2016b). This is an intriguing finding indicating a hitherto overlooked source for PCB pollution. A study from northern Italy is reporting on abundant decachlorobiphenyl levels in soil but not of nona- and octaCBs (Bagnati et al. 2019) indicating at least that PCB production could be a source also for the pollution in the wildlife from the YRD.
Further, a whole group organochlorine pollutant of still unconfirmed identity was detected in the heron and tern eggs analysed (Zhou et al 2016b). The figure 4 presented by Zhou and co-workers is implying that the group to consists of one octa-, three hepta-, five hexa- and one pentachlorinated congener. We speculate and believe that the most likely identity of this novel pollutants is polychlorinated stilbenes of the general structures as proposed in Figure 3.6.5. If it is polychlorinated stilbenes we still have to consider if these are cis- and/or trans-stilbenes. We are at present excluding another possibility since it is based on a DDE related chemical structure, still acknowledging this being a possibility.

Methodological development has brought us to a position when it is now possible to present a method for analysis of the pesticide, dicofol, a compound applied as a substitute for DDT (Yin et al. 2017). With this method, it is possible to quantify dicofol and dichlorobenzophenone in the same samples.

The data obtained as described above calls for continues monitoring of some of the selected species, to follow the development of their concentrations in wildlife, in the future. The work presented herein is strongly linked to what is presented in the next chapter, 3.7 related to pollutants in herons. Chemical analysis of heron eggs seem to be a good choice for future monitoring.

Finally, the text above is only an extract of results from project 3.6. Hence consult the full list of original articles that have come out from the project as they are listed below.

**Key message/Implications**

- The screening of POPs in wildlife from the YRD has been successful, showing that the chemical analysis based on pollutants chemical properties makes it possible to identify emerging and novel pollutants in the environment (wildlife) but will also work for human samples.
- The high concentration of CPs in wildlife samples from the YRD suggest that action should be taken in order to manage the contamination situation. It is clear that also MCCP and LCCP must be included in the Stockholm Convention as POPs.
- A novel group of persistent and bioaccumulative chemicals has been indicated in wildlife.
- Highly chlorinated biphenyls, of unknown origin, were identified and quantified.
- Further monitoring of POPs in the YRD need to be done for years to come in order to determine trends in pollution levels.
- We propose eggs from one identified heron species to be applied in the YRD monitoring program and that eggs from this species is to be sampled for the Yangtze ESB.

**Scientific Publications from the subproject**


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**References**
Objectives
The aims of this project were to develop heron egg as a bioindicator for chemical pollution in the Yangtze River Delta, which is needed for the assessment of environmental quality and for assessing potential concerns for human health. Our objectives were:
– to identify a suitable species among herons for long term monitoring of chemical contaminants and for collection of material to the Yangtze environmental specimen bank (YESB).
– to assess trends and status of known chemicals as well as novel emerging environmental contaminants in bird eggs.
– to identify suitable sample sites for a long-term biomonitoring program with bird eggs.

The project workflow is visualized in Figure 3.7.1.

Main results
Persistent organic pollutants (POPs) have been released into the environment due to their widespread use and inherent physicochemical properties. They are fulfilling the Stockholm Convention for POPs, i.e. being persistent, bioaccumulative, toxic and with the capacity of long-range global transport (Bilcke, 2003). The properties of the POPs cause risks to wildlife (e.g. invertebrates, fish, aquatic mammals and birds) as well as for human health and wellbeing worldwide. In Europe and America, bird eggs have been used for biological monitoring of pollutants for several decades (c.f. the Swedish environmental monitoring program, www.swedishepa.se).

Successful attempts to confirm the relationship between pollutants and population reduction of wildlife have been determined. Taking the black-crowned night heron (Nycticorax nycticorax) as an example, it was once found that its fecundity was low among the herons in the mountains of the western United States. Later, it was found to be related to the egg shell thinning caused by the high concentration of DDE in the eggs of the night herons (Henny et al., 1984).
Figure 3.7.1. Illustration of the project workflow.
With the reduction of DDE and PCBs in the eggs of night heron, the egg shell thickness increased, and populations have recovered just as shown among birds of prey in Sweden (Bignert et al. 1995, Bignert & Helander, 2015).

Although the levels of organochlorine pesticides (OCPs) in waterfowl tissues (especially in the eggs of night heron) has been decreasing in the past 20 years, DDE and PCBs still show sublethal effect and influence on reproduction in some sampling sites. OCPs and their metabolites are accumulated in the nestlings of night heron. As we know, a vast majority of existing and new potential POPs are not monitored in environmental media (Muir & Howard, 2007). Therefore, bird eggs can be effectively used to screen POPs residues and monitor the trend of its exposure and effect.

**Occurrence of POPs and emerging contaminants in bird eggs**

Egg samples of little egret (Egretta garzetta), night heron (Nycticorax nycticorax), Chinese pond heron (Ardeola bacchus), cattle egret (Bubulcus ibis) and whiskered tern (Chlidonias hybrida) were collected from Chongming Island, Taihu Lake and Tianmu Lake in the Yangtze River Delta and in the Yibin area in the upper reaches of the Yangtze River (ca. 2800 km straight west of the Yangtze river delta). Traditional POPs and emerging organic contaminants were analyzed. The results are presented below.

The concentrations of OCPs, PCBs, PCDD/Fs and PBDEs have been determined in eggs from several bird species (Zhou et al. 2017 and 2016), including the black-crowned night heron and whiskered tern (Figure 3.7.2). OCPs and their metabolites are common in bird eggs, but the residual levels of different chemicals are quite different. The concentration of DDE is the highest, followed by...
β-HCH and Mirex. Compared to other places in the world (Figure 3.7.3), the levels of OCPs in night heron eggs from the Yangtze River basin are lower, not sufficient to affect its reproduction. High chlorinated biphenyls with 8-10 chlorine atoms accounted for about 10% of the total PCBs in bird eggs (Zhou et al. 2016). The occurrence of highly chlorinated biphenyls is discussed in Chapter 3.6. However, this novel pattern of PCBs in wildlife need further attention in order to identify their sources.

Further, octa-, hepta- and hexachlorodibenzo-p-dioxin are abundant in eggs from black-crowned night heron and whiskered tern, indicating that pentachlorophenol (PCP) is an important precursor of PCDD (Zhou et al. 2017).

Chlorinated paraffin (CPs), polychlorinated diphenyl ethers (PCDEs) and their derivatives are present in heron eggs (Zhou et al. 2016; 2017). Herein, the occurrence of organophosphorus flame retardants (OPFRs) is discussed in some further detail. OPFRs are substitute chemicals for brominated flame retardants (BFRs), OPFRs were detected in all kinds of heron eggs in the Yangtze River Delta and its upper reaches (Figure 3.7.4). In general, tri-n-butyl phosphate (TNBP), tris(isobutyl) phosphate (TIBP), tris(1-chloro-2-propyl) phosphate (TCIPP) and tris-2-methylphenyl phosphate (TMPP) are the most abundant OPFRs. Among them, TMPP, TCIPP and TBOEP are embryotoxic, which may have an impact on reproduction and deserve attention (Crump et al. 2012; 2014; Greaves & Letcher, 2014; Porter et al. 2014). In addition, taking OPFRs as an example, the night heron eggs seem to deviate from the eggs of other heron species, and the OPFRs levels of night heron eggs are significantly higher than those of little egret eggs and pond heron eggs.

**Linkage between chemical pollution level in heron eggs and regional environment**

With the development of social economy, the degree of industrialization and urbanization in the Yangtze River Delta and other developed regions of the world is increasing. Various chemicals of widespread use are gradually released into the environment, causing water pollution. Persistent, bioaccumulative and toxic substances can enter the organisms through drinking and feeding, and transfer along the food chain (Figure 3.7.5), finally bring risks to wildlife (e.g. invertebrates, fish, aquatic mammals and water birds).
and human health. Birds are top consumers in the food chain. POPs including OCPs, PCBs and OPFRs in the female birds can be delivered to their eggs.

The concentration of OPFRs in heron eggs from downstream Yangtze River, at Chongming was significantly higher than that in heron eggs from upstream at Yibin, and the distribution pattern of OPFRs was also different. The Yangtze River Delta has more developed economy and faster industrial development than the upper river system, which may explain the geographical difference of OPFRs. Despite this, with the progress of urban renewal and the upgrading of ecological environment protection in developed areas in China, the pollution levels of pesticides and industrial chemicals in heron eggs are expected to decrease.

**Potential health risks of wild bird egg consumption**

Generally, the nutritional value of wild bird eggs is considered to be higher than that of poultry eggs.
Accordingly, people may take the opportunity to collect wild bird eggs for their own consumption or serving in restaurants. Does it cause human health risks? Taking heron eggs as an example, we evaluated the dietary exposure to dioxins (traditional POPs) and OPFRs (emerging contaminants) in the eggs of night herons in Taihu Lake basin.

The toxic equivalent factor (TEQ) of PCDD/Fs in the eggs of night heron in Taihu Lake Basin far exceeds the regulation limit (2.5 pg TEQ g\(^{-1}\) lw) of European Union on poultry eggs and egg products. According to the tolerable intake (14 pg TEQ g\(^{-1}\) bw/week), these eggs will cause health concerns to regular consumers, especially children (Zhou et al. 2017).

For the emerging contaminants OPFRs, the daily intake level via ingesting heron eggs is far lower than the reference dose (RfD), which is not likely to cause adverse effects on health. However, it can be compared with the intake of drinking water or seafood consumption and other ways when it is frequently consumed (e.g. 1 egg/day).

Further, the combined exposure to mixtures of pollutants need to be considered (Kortenkamp & Faust, 2018). In this case, measures should be taken immediately to limit the consumption of heron eggs, and further study and evaluate the potential health effects of the consumption of wild bird eggs in China. It is also necessary to study the level of dioxin pollution and its health risk in common eggs in China. In fact, a preliminary study of commercial chicken and duck eggs has taken place and the results from that study indicate very low levels of POPs in the chicken and duck eggs (unpublished). Based on the data obtained regarding heron eggs we want to put forward the recommendation presented in Figure 3.7.6.

**Key message/Implications**
- Persistent, bioaccumulative and toxic substances can be detected in eggs from different species of herons.
- The eggs of night herons can be used in biomonitoring of contaminants.
- Heron eggs from Chongming Island and the Yibin area can be used as sampling sites for long-term monitoring.
- Please inform people not to eat heron eggs.

**Scientific Publications from the subproject**

Researchers involved in the project
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References
3.8 Mothers’ milk monitoring in the Yangtze River Delta

Objectives
Women in child carrying age, the foetus, the newborn baby and children are regarded as the most vulnerable humans for exposure to environmental contaminants. Hence there are in particular reason to minimise their exposures. Mothers’ milk is recognized as an important matrix for monitoring of both spatial and temporal trends, worldwide. Still it is rare with longer lasting monitoring programs on national basis.

The objectives of the present work are:
– to establish a scientifically based structure for sampling and storing of mothers' milk for the Yangtze ESB (Environmental Specimen Bank).
– to design a future monitoring program in the Yangtze River Delta.
– to analyze and report temporal trends and geographical differences of persistent and bioaccumulative organic contaminants in milk samples, collected during the period.

In the beginning of the project, geographical comparisons were made between various cities in China and between China and Sweden for legacy POPs like PCBs, DDTs, HCHs and HCB. A manuscript was recently prepared and submitted on Chlorinated Paraffins (CPs) including mothers' milk from China, Sweden and Norway and another manuscript is under way for per- and polyfluoroalkyl substances (PFASs).

Main results
Monitoring of environmental contaminants is typically carried out with samples from wildlife e.g. fish or invertebrates in aquatic ecosystems. However, one major concern is how the environment affect human beings. The borders between scientific disciplines like medicine, environmental chemistry, biology and food science are floating and many environmental agencies around the world, wisely include mothers' milk in their traditional environmental monitoring programs. WHO is
running a mothers' milk program since 1976, Sweden since the late 60’ies and China from 2007. A review article (Fång et al. 2015) summarized a vast number of POP assessments in mothers' milk.

Like any other monitoring matrix, concentrations in mothers' milk are affected by several factors that from an environmental perspective can be considered as confounding factors. That is, the measured concentrations do not only reflect changes in exposure from e.g. food but also factors like the mother’s age, (increasing concentrations at higher age, Albers et al. 1996), number of children (concentrations in the mother decreases after child birth, Fitzgerald et al. 2001, e.g. chlorinated paraffins concentrations in the YRD samples from first-time mothers were on average three times higher than from second-time mothers (Zhou et al. 2019), BMI (body mass index, decreasing concentrations at higher BMI), weight gain during pregnancy (decreasing concentrations) (Nyberg et al. 2016). Furthermore, economic situation, ethnicity, education and occupation (e.g. fisherman’s wives) may affect food habits. It is of course important to distinguish between different purposes with the monitoring. From an environmental point of view the factors listed above are confounders that needs to be addressed in order to get more unbiased results e.g. temporal trend estimates from the monitoring but for the newborn baby the measured concentration reflects the actual exposure.

The previous summary report (Chapter 3.8 and Figure 3.8.1 in Bergman et al. 2017 and Fång et al. 2015) showed major differences between cities in China of legacy POPs like DDTs, HCHs, PBDEs. Also, a comparison between China and Sweden points to considerably higher concentrations of PCB (CB-153) in Sweden compared to China but substantially higher concentrations of DDTs, HCHs and also HCB in China compared to Sweden. The indicated concentrations of DDTs and HCHs were surprisingly high considering that the use of these chemicals was banned quite a long time ago. Banned pesticides like HCHs and DDTs and industrial chemicals like PCBs, but also unintentionally produced dioxins, have decreased substantially in mothers' milk from Sweden during recent decades (Nyberg et al. 2018).

A recent study analyzed short-, medium-, and long-chain chlorinated paraffins (SCCPs, MCCPs, and LCCPs) in mothers' milk from the Yangtze River Delta (YRD) and from Scandinavia (Zhou et al. 2019). Individual samples were collected from Shanghai, Jiaxing, and Shaoxing (China), Stockholm (Sweden), and Bodø (Norway), between 2010 and 2016. The sampling was, with some minor exceptions, based on the WHO guidelines for human milk monitoring (WHO, 2007). Concentrations of S/M/LCCPs in samples from YRD were all significantly higher than in samples from Scandinavia (p<0.05). Herein a comparison is shown on the S/M/LCCPs in mothers milk sampled in Shanghai and in Stockholm (Figure 3.8.1). This study is the first to show LCCP exposure via mothers' milk. It furthered showed that the YRD samples were more influenced by SCCPs than the Scandinavian samples, reflecting different exposure to CPs between regions (Figure 3.8.1). Individual samples enabled estimates of within-population variation and indicated that SCCP intake poses a potential risk for Chinese infants, exceeding the 90th percentile. It is the subdivision of the CPs into three classes that give an impression of similar concentrations to some other POPs. However, if the CPs are presented as one class these are the pollutants with the highest concentrations together with PFAS. The latter are structurally more different than the whole class of CPs.
In another recent study, PFASs were determined in samples of mothers’ milk collected between 2010 and 2016 from three Chinese cities (Shanghai, Jiaxing, and Shaoxing) (Awad et al. 2019). These data were compared to previously reported PFAS data from mothers’ milk from Stockholm, Sweden, sampled in 2016 (Nyberg et al. 2018) and also with studies from China (e.g. Tian et al. 2018, Lui et al. 2010). In all of the studied Chinese cities, perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS) occurred at the highest concentrations, in Stockholm, PFOA and PFOS were the dominant PFASs (Figure 3.8.1.).

![Figure 3.8.1. Box and whisker plot (25th and 75th percentile) showing examples of mothers’ milk contaminants, i.e. short, medium and long chain chlorinated paraffins (SCCPs, MCCPs and LCCPs), perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), 2,2’,4,4’-tetrabromodiphenyl ether (BDE-47), 2,2’,3,3’,4,4’-hexabromodiphenyl ether (BDE-153), β-hexachlorocyclohexane (β-HCH), hexachlorobenzene (HCB), 1,1'-bis(4-chlorophenyl)-2,2-dichloroethene (DDE) and 2,2’,3,3’,4,4’-hexachlorobiphenyl (CB-153). The concentrations (ng g⁻¹ fat) are presented in ng g⁻¹ fat in the exponential scale to enable us to show the concentrations ranges for all the selected analytes. The human milk samples were collected from mothers living in Shanghai (China) and Stockholm (Sweden, 2011 and 2016), (Zhou et al. 2019 and Awad et al, 2019).](image1)

In Sweden PFOS concentrations in mothers’ milk are generally decreasing, in China however, where PFOS production continued until 2017 under the Stockholm Convention manufacture and use exemptions, no declines in PFOS have been observed. The report further concludes that human exposure assessments focused only on legacy substances may severely underestimate the overall exposure to PFASs. The total number of different PFAS in use today counts to around 4500 individual chemicals.

The present study is, as visualized in Figure 3.8.1, confirming high concentrations of DDE, originating from DDT, and β-hexachlorocyclohexane (β-HCH) showing similar concentrations to the SCCP, MCCP, PFOA and PFAS in the mothers’ milk from Shanghai. This is also true for these compounds when compared with milk from Stockholm mothers. The concentrations of the two polybrominated diphenyl
Ethers (BDE-47 and BDE-153) are the lowest with rather similar concentrations in the milk from Shanghai and Stockholm mothers. The reverse, as discussed above, is the case for the polychlorobiphenyl CB-153, where the concentrations in mothers’ milk from Stockholm is significantly higher than in milk from Shanghai mothers’ milk.

The study shows how complex the mixture exposure is for not only the nursing children but also the exposure during the fetal period. These are all compounds that can be transferred over the placenta. Steps need to be taken to manage exposure to mixtures as recently discussed by Drakvik et al. 2019.

**Key message/Implications**
- Mothers’ milk is an important sample matrix to assess exposure of organic contaminants to nursing children and can also reflect general changes over time and geographical differences but should also contain information about potential confounding factors (i.e. the WHO guidelines should be followed). Individual samples instead of pooled samples enable estimates of distribution within a population, that is imperative to assess exposure in segments of the population at potential risk. Pooled samples may still be a cost efficient alternative to study general temporal trends or geographical differences.
- Further studies on banned pesticides are required to see whether the bans have been efficient.
- The global ban of SCCPs as POPs needs to be followed up, to include also MCCPs and LCCPs.
- PFAS are present in high concentrations as exemplified above and accordingly exposures need to decrease through adequate management.

**Publications from the subproject**
- Yihui Zhou, Bo Yuan, Elisabeth Nyberg, Ge Yin, Anders Bignert, Anders Glynn, Jon Øyvind Odland, Yanling Qiu, Yaije Sun, Yongning Wu, Qianfen Xiao, Daqiang Yin, Zhiliang Zhu, Jianfu Zhao, Åke Bergman (2019). Chlorinated Paraffins in Human Milk from Urban Sites in China, Sweden, and Norway (Submitted for publication)
- Raed Awad, Yihui Zhou, Elisabeth Nyberg, Yongning Wu, Qianfen Xiao, Yaije Sun, Zhiliang Zhu, Åke Bergman and Jonathan P. Benskin. (2019). Emerging Per- and Polyfluoroalkyl Substances (PFASs) in Human Breast Milk from Sweden and China (Manuscript)

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**References**
3.9 Establishment of optimal fish species for research monitoring in the Yangtze River Delta

**Objectives**
Life below water as habitat for an uncountable number of plants and wildlife that must be protected. To promote a sustainable environment in the aquatic environments it is very relevant to study fish. The achievements of the present project will provide a foundation for monitoring the environmental quality in YRD. The objectives of the project have been:

- to find optimal fish species for environmental monitoring in the Yangtze River Delta (YRD).
- to identify good candidate species that include a combination of both chemical analysis and biological effect monitoring possibilities.

**Main results**
Environmental monitoring is performed by measuring the indices that are indicative for the environmental quality, and is aimed to determine the status of environmental pollution and the level of environmental quality. It involves monitoring physical, chemical and biological/ecological indicators. Various species of fish is commonly used within Europe and North America to monitor chemical status and long-term changes in the environment. It can give information of the bioavailable proportion of discharged environmental contaminants and can also help in human risk assessment since fish is used for human consumption. Yangtze River Delta (YRD) is one of the most economically developed areas in China. The usage of chemicals is increased with the development of economy. It is necessary to carry out environmental monitoring to control the quality of water environment. YRD have a developed water system and rich fishery resources but lack such a fish biomonitoring project.

To solve this question, the present project focused on two groups of chemicals, in particular polybrominated diphenyl ethers (PBDEs) and short chain chlorinated paraffins (SCCPs). Both chemicals are listed as Persistent Organic Pollutant (POP) in the Stockholm Convention (UN Stockholm Convention, 2019). We collected wild fish from Dianshan Lake in YRD
to perform chemical analysis to demonstrate the distribution pattern of the chemicals. Laboratory research on model fish was also carried out to indicate potential toxic hazards and health risk.

The fish species included were: Grass carp (*Ctenopharyngodon idellus*), Bighead carp (*Aristichthys nobilis*), Silver carp (*Hypophthalmichthys molitrix*), Crucian carp (*Carassius auratus*), Common carp (*Cyprinus carpio*), Snakehead (*Channa argus*) and Yellow catfish (*Pelteobagrus fulvidraco*). To choose appropriate fish species for monitoring, the fish that have the greatest abundance were considered to ensure their representative roles to indicate the environmental quality. For example, Grass carp, Crucian carp, and Yellow catfish are the main economic fish species in Taihu Lake, Qiangtang River, Downstream of Yangtze River, Hongze Lake, Tianmu Lake and Yangcheng Lake. Bighead carp widely live in Taihu Lake, Qiangtang River, downstream of Yangtze River and Tianmu Lake. Silver carp lives in Qiantang River, Hongze Lake, Tianmu Lake and Yangcheng Lake. Common carp widely exist in Qiangtang River and Hongze Lake, and Snakehead are often caught in Dianshan Lake.

Assessing pollutant concentrations show the highest mean concentration of ∑7PBDEs in snakehead (38 ng g⁻¹ lw) whereas the lowest concentration was observed in grass carp (0.26 ng g⁻¹ lw). Mean concentrations of PBDEs were in the following descending order: snakehead > stone moroko ≈ yellow catfish ≈ bigmouth grenadier anchovy > rosy bitterling ≈ common carp > predatory carp ≈ crucian carp ≈ silver carp ≈ bighead carp > grass carp (Du et al. 2017; Zhou et al. 2016). In general, the contaminant levels in fish from Dianshan Lake were in moderate to low range compared with other studies (Figure 3.9.1). The PBDEs concentrations were lower than or similar to those in some waterbodies in southern China and those in Taihu Lake, and they were much lower than those in Gila River, USA.

Concentrations of SCCPs in aquatic species ranged from 10 to 1300 µg g⁻¹ lw in our study (Du et al. 2018; Zhou et al. 2018). The highest levels of SCCPs were detected in common carp, with a mean concentration of 280 (range: 40-1300) µg g⁻¹ lw, followed by crucian carp (mean: 220 µg g⁻¹ lw, range: 35-650 µg g⁻¹ lw), Figure 3.9.2. The lowest level was found in stone moroko, with the mean concentration of 14 (range: 10-17) µg g⁻¹ lw. In general, the concentrations followed the descending order as: common carp > crucian carp > yellow catfish > bighead carp > snakehead > silver carp ≈ shrimp > bigmouth grenadier anchovy > rosy bitterling ≈ grass carp > stone moroko. Concentrations of SCCPs in benthic organisms (150 µg g⁻¹ lw in average) were significantly higher than those in non-benthic fish.
Chapter 3: Chemstrres research: Main results and messages

(56 µg g⁻¹ lw in average) (p<0.05), indicating accumulation of SCCPs to a higher extent in bottom-dwelling organisms. Notably, the accumulation was greater in wild fish than farmed, which could be due to the size dilution in the latter which grew faster with higher lipid levels. The SCCPs levels in fish from Dianshan Lake were similar to those in Liaodong Bay and Bohai Sea, while higher than the aquatic organisms in Norway, Canada and Spain. Such difference might due to the extensive production and usage of CPs in China.

Zebrabox (Viewpoint, France) was used to build three neurobehavioral analysis methods of larval zebrafish larvae, including locomotion, path angle and social activity. Then neurobehavioral effects caused by BDE-47 in three exposure modes (continuous exposure, early pulse exposure and interval exposure) were studied (Zhao et al. 2014; Zhang et al. 2017), and neurobehavioral effects of 6-OH/MeO-BDE-47 were compared (Zhang et al. 2018). Results in the aforementioned studies showed that the highest dose of BDE-47 elicited pronounced hypoactivity at 5 days post fertilization (dpf) during dark periods in the continuous exposure and interval exposure mode. However, at 6 dpf, hypoactivity was only observed in the continuously exposed zebrafish larvae, but not in the interval exposure treatment group, suggesting that high concentration of BDE-47 exposure could induce neurobehavioral toxicity and locomotor effects on PBDE exposure depending on the specific exposure mode studied. For the path angle, BDE-47 caused less straight and average turns but more responsive turns; light influenced responsive turns and social behavior of zebrafish larvae during the interval exposure mode. High concentration BDE-47 treatment group influenced social behavior, and the influence grew with increasing exposure time. The different exposure modes of BDE-47 yielded different neurobehavioral effects, i.e. continuous exposure > early pulse exposure > interval exposure.

Results showed that the two OH-PBDEs did not induce significant effects on larval locomotion. 6-OH-BDE-47 mainly decreased average and routine turns, but 6-MeO-BDE-47 promoted responsive turns of larvae (one-way ANOVA, p < 0.05). Different effects of the two derivatives on path angle may indicate their different toxicity mechanism. They both inhibited social activity but only the effects of 6-MeO-BDE-47 had significant difference.

Optokinetic response (OKR) and looming stimuli were used to evaluate visual effects of BDE-47 exposure on larvae. BDE-47 exposure significantly reduced the larval OKR response to the blue light but not the mid-long wave-length lights (one-way ANOVA, p < 0.05). Additionally, larval left eyes had fewer movements than the right eyes after BDE-47
exposure, while wild-type larvae had balanced movements of two eyes to different colors. We also investigated the saccadic movement angles per time using the VisioBox; however, no significant difference was observed between the control and BDE-47 treatment (Xu et al. 2017). In the looming results, the amounts of responsive fish significantly reduced with BDE-47 exposure, reflecting BDE-47 impaired larval behavioral capacity with sensing ambient visual stimuli. Furthermore, larval response time to stimuli were not retarded; the relationship between response time and visual angles, which was commonly used in studies of looming-evoked visual pathway mechanisms, were also not influenced (Xu et al. 2017). The effects existed only in response rate, not action mode nor response speed.

Increasing concern has arisen regarding ubiquitous environmental distribution and potential ecological and health risks of CPs, especially SCCPs. Four commercial CP products with different carbon chain lengths and chlorine contents were employed to investigate and compare the possible neurotoxic effects on zebrafish larvae at 5 days post fertilization using behavioral tests, including locomotion, path angle, and two-fish social interactions (Yang et al. 2019). The high-chlorinated short-chain CP-70 product resulted in the strongest effects in all three tests, while the low-chlorinated long-chain CP-42 product was on the other end of the spectrum. The consequences of the chain length of two CP-52 products could be clearly distinguished by the tests. Although exposure to short-chain CP-52b caused more inhibition in the locomotion test than CP-52a did, the two products resulted in different kinds of effects in the path angle and interaction tests. Results suggested, as evidenced by the sensitivity and resolution of the behavioral tests, that the influence of the chain length and chlorine content of CPs could be well characterized and that chlorine content consistently showed a more significant impact than chain length. The health threats of long-chain CPs could also not be overlooked when they contained relatively high chlorine contents.

**Key message/Implications**

- The wild fish results demonstrated that the local abundant fish including yellow catfish accumulated PBDEs and SCCPs and were considered optimal fish species for the bio-monitoring in YRD.
- The lab fish results demonstrated that neurobehavioral indicators were sensitive to PBDEs and SCCPs.
- The neurobehavioral test kept the fish intact and facilitated later chemical analysis. Such combination will provide foundations for an environmental monitoring integrating both biological and chemical indicators.

**Scientific Publications from the subproject**

- Zhou Y, Yin G, Du X, Xu M, Qiu Y, Ahlqvist P,
– Zhou Y, Asplund L, Yin G, Athanassiadis I,


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References
3.10 Chemical pollutants in dust

Objectives
Household dust is a mirror of which chemicals are applied in construction and decoration materials as well as of the goods in our homes. Hence further focus is required for development of safe materials for building sustainable cities and consumables. Discharges of chemicals to dust will potentially influence the human health and wellbeing. Accordingly, studies to better understand dust exposures their importance and needs for management.

The present subproject of Chemstrres has addressed the following objectives:
– to determine concentrations and compositional profiles of chemical pollutants in floor dust from home environment in Shanghai and Stockholm.
– to determine seasonal variation in concentrations due to sampling period of the year.
– to explore the main sources of chemical pollutants in home environments.
– to estimate human health concern in association with floor dust intake for Shanghai residents.

Main results
Particle size distribution of household dust and occurrence of several groups of organic pollutants in various particle size fractions. Dust collection from 10 Shanghai homes was carried out in April 2017. The dust from commercial air cleaner bags were pooled and separated prior to analysis. The dust collection from Stockholm houses were conducted (Gustafsson et al. 2018) in parallel. The fractionation method was the same independent of the household dust was sampled in Shanghai or Stockholm. Organic flame retardants containing phthalates (PAEs), chlorinated paraffins (CPs), organophosphate flame retardants (OPFRs), polybrominated diphenyl ethers (PBDEs) and novel brominated flame retardants (NBFRs) were examined in fractions of Shanghai household dust (Figure 3.10.1). In Stockholm dust fractions, only OPFRs were analysed. In Shanghai household dust, total concentrations ranged from 588 to 1940 µg/g (median: 918 µg/g) for PAEs, 375 to 724 µg/g (median: 467 µg/g) for CPs, 5.3 to 14 µg/g (median: 7.2 µg/g) for OPFRs, 0.36 to 5.0 µg/g.
(median: 0.73 µg/g) for PBDEs and 0.088 to 0.17 µg/g (median: 0.16 µg/g) for NBFRs. Statistical analysis showed that PAEs concentrations were significantly higher than PBDEs concentrations (p < 0.05) and NBFRs concentrations (p < 0.01), and CPs concentrations were significantly higher than NBFRs concentrations (p < 0.05). In general, PAEs concentrations were three times that of CP concentrations, 300 times that of OPFRs concentrations, 2000 times that of PBDEs concentrations and 9000 times that of NBFRs concentrations. These results mirror to quite some extent the production volumes in China where PAEs and CPs by far exceed that of PBDEs and NBFRs. According to literature review, PAEs was produced 1.4 million tonnes in 2014 (Zhu et al. 2019), CPs 1.05 million tonnes in 2013 (Du et al. 2019), OPFRs 100000 tonnes in 2011 (Wang et al. 2015), Deca-BDE (the dominating congener of PBDEs) 20,500 tonnes in 2011 (Ni et al. 2013) and Decabromodiphenyl ethane (DBDPE), the dominating compound of NBFRs in dust, in 25000 tonnes in 2012 (Wang et al. 2018).

Bis(2-ethylhexyl) phthalate (DEHP) dominated among the PAEs, accounting for 79-85 % in dust fractions. Medium-chain chlorinated paraffins (MCCPs, C14-17) was the major group of CPs, with relative abundance from 42-50% in dust fractions. Chlorinated OPFRs (the sum of tris(2-choroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP) and tris(1,3-dichloroisopropyl) phosphate (TDCIPP)) (42-74%) and deca-BDEs (80-87%) dominated in all fractions. For NBFRs, the predominant compounds were different in fractions, with DBDPE the most abundant 47% in F3, 2-ethylhexyl 2,3,4,5-tetabromobenzene (EHTBB) 59% in F1, 49% in F4 and 50% in F5, and 1,2-bis(2,4,6-tribromophenoxy) ethane (BTBPE) 63% in F2.

The concentrations of the pollutants on the dust related to the particle size in Shanghai dust samples are presented in Figure 3.10.1. The distribution patterns indicated that the highest concentrations were observed in the finest fraction which is the respirable fraction (F5e) for PAEs, OPFRs and PBDEs. For the CPs and NBFRs the peak concentrations were observed in the middle size dust fraction (F3).

In Stockholm household dust fractions, OPFRs ranged from 16 µg/g to 28 µg/g (median: 27 µg/g), which is significantly (p < 0.01) higher than the levels found in Shanghai household dust. The composition profile between the two cities was also different (Figure 3.10.2). Chlorinated OPFRs, commonly applied as flame retardants, were the major compounds in dust from Shanghai homes, whereas tris(2-butoxyethyl) phosphate (TBOEP), which is used in floor polish, dominated in dust from Stockholm homes.
Figure 3.10.2. Compositional profile of OPFRs in the following fractions, (F1: 190-390 μm; F2: 75-190 μm; F3: 25-75 μm; F4: 4-25 μm; F5: < 4 μm) in household dust from Shanghai (Sh) and Stockholm (St).

**Brominated flame retardants in floor dust (FD) and elevated surface dust (ESD).** A total of 154 dust samples, including FD and ESD, were collected from 22 dwellings every second month in the biggest metropolitan area (Shanghai) of East China in 2016 (Figure 3.10.3.). Household dust was separately collected from the floor and elevated furnishings surface (mostly between 0.5 and 2 m height). All dust samples were wrapped in solvent-cleaned aluminum foil and then sealed in polyethylene bags before taken back to the laboratory.

Novel brominated flame retardants have been increasingly used as alternatives to PBDEs in consumer products, but able to migrate and contaminate indoor dust. Levels, temporal variation, and human exposure of PBDEs and NBFRs such as DBDPE, BTBPE, EHTBB, bis (2-ethylhexyl) tetrabromophthalate (BEHTEBP), hexabromobenzene (HBB), pentabromotoluence (PBT) and pentabromoethylbenzene (PBEB), were investigated for their occurrence in indoor household dust.

In terms of PBDEs, 22 PBDE congeners were selected for analysis, and the concentration of Σ22PBDEs in FD was comparatively higher than that in ESD. Compared with some European and North American countries, the concentrations of PBDEs in this study were relatively low (Niu et al. 2018). DecaBDE (BDE-209) was the predominant PBDE congener, accounting for about 73% and 55% in FD and ESD respectively (Figure 3.10.4.). The high abundance of BDE-209 in house dust is probably due to massive use of products containing BDE-209 and its low volatility, which makes it liable to be found on particulates at room temperature. As the secondarily abundant homolog, the nonaBDEs (BDE-206, -207, -208) contributed 6-36% of the total PBDE burden except in three samples of one apartment where it was >40%. The results showed that BDE-85, -153,
-183 and -209 in FD exceeded significantly those in ESD. One possible explanation for the results is that the majority of floating dust particles, which PBDEs adhered to in the air, eventually drifted down and accumulated on the floor. Another possibility is that PBDE concentrations were negatively correlated with surface loadings (ng dust per m² of floor area). Relative higher dust loadings in ESD samples resulted in lower levels of PBDEs, “dilution” occurs at higher dust loadings. The comparison of FD and ESD revealed no significant differences except for the ratio of BDE-47/BDE-99. ESD samples displayed a ratio of BDE-47/BDE-99 very similar to the commercial PentaBDE mixture, DE-71, whereas FD samples displayed an aberrant ratio compared with commercial products. In addition, significant correlation was found between concentrations of commercial PentaBDE compositions in FD and ESD ($p < 0.05$).

With respect to NBFRs, DBDPE, as the substitute for DecaBDE, clearly dominated the NBFRs in both FD and ESD, accounting for 90% and 80% of $\Sigma_7$NBFRs in FD and ESD respectively (Figure 3.10.4). However, the concentrations of DBDPE in this study (Niu et al. 2019) were generally moderate compared with the other international studies. Compared with DecaBDE in this study (Niu et al. 2018), DBDPE concentrations rivaled those of DecaBDE, revealing that DBDPE has been widely used in China following the phase-out of PentaBDEs and OctaBDEs.

BTBPE, as a replacement for OctaBDEs in the ABS polymer, thermoplastics and textile applications, was the second most abundant NBFRs. The full detection frequency of BTBPE indicates the extensive use of this emerging contaminant in indoor environment. Compared with other international studies, the
concentrations of BTBPE presented in this study (Niu et al. 2019) were of middle level. The difference of NBFRs between FD and ESD was compared testing whether it was necessary to separately collect two dust categories for analyzing NBFRs, and then for further estimating human exposure risks in different age groups. The results showed that concentrations of EHTBB, BTBPE and DBDPE in FD exceeded significantly those in ESD. Spatial variations in BFR concentrations were probably driven by various potential emissions or abrasion sources from household products. Owing to possible abrasion of household products containing NBFRs that causes precipitation to the floor by gravity, and the low vapor pressure that may facilitate partitioning to the FD. The EHTBB, BTBPE and DBDPE seemed to be more abundant on the floor than on elevated surfaces of household products in indoor environment, but it is not fully true. Multiple mechanisms might influence the fate of each NBFR compound. Besides, strongly significant correlations of HBB, BEHTEBP and DBDPE were found between FD and ESD (p < 0.01). The results indicated that these compounds might be from similar sources, i.e. polymeric materials.

PBDE levels exhibited generally temporal stability over the year, in this study (Niu et al. 2019). The temporal concentration variations of PBDEs between the maximum and minimum were approximately twofold, with the highest in August and lowest in April.

Increasing/decreasing emissions of PBDEs in warmer/colder months would probably be counterbalanced by indoor air conditioning. The temporal variation of Σ_{7}NBFRs in FD was ranked as summer > winter > autumn > spring. Higher room temperatures in the summer may be one possible explanation for higher concentrations of NBFRs, potentially coming from e.g. electrical appliances and furnishings. Interestingly, FD in winter showed the second highest concentration of Σ_{7}NBFRs. Household heating system indoors may also counterbalance the effect of lower temperature in winter. Moreover, ventilation in winter is poorer, which may contribute to the residence of NBFRs indoors. In conclusion, it is not yet understood how the emissions occur over the year and indoors.

The human intake can occur from inhalation, dermal contact, and dust ingestion. In domestic indoor microenvironment, dust inhalation/ingestion are main routes of human exposure to PBDEs and NBFRs, particularly for toddlers, due to their hand-to-mouth behavior, who are deemed to be more susceptible. In this study, about 20-fold and 8-fold difference in PBDEs and NBFRs exposure, respectively, estimates between toddlers and adults supports that toddlers are facing greater risk from chemicals adsorbed to indoor floor dust. The estimated non-carcinogenic and carcinogenic risk levels of PBDEs for toddlers and adults were both below the safe limits of U.S. Environmental Protection Agency, indicating that people in this study have low-dose exposure risks when only neurobehavioral effects were considered. The daily exposure doses of four main NBFRs (EHTBB, BEHTEBP, BTBPE and DBDPE) via dust ingestion were also estimated for exposure risk from single NBFR compound. Compared with oral reference doses (RfDs), the estimated daily exposure doses of EHTBB, BEHTEBP, BTBPE, and DBDPE were at least 2×10^6, 10^6, 3×10^6, 3.3×10^6 times lower in this study, probably revealing that residents faced low exposure risks from these four NBFR compounds in indoor house dust. We assumed 100% absorption efficiency of intake, but this assumption may result in the overestimation of human exposure to BFRs. People may face lower risks if bioaccessibility of BFRs is taken into account in future studies.
More work need to be done for human exposure to PBDEs and NBFRs in indoor dust via ingestion, inhalation, and dermal contact which would directly or indirectly affect the lifetime risk of cancer. This study highlighted the necessity to estimate human exposure of BFRs for different age groups exposed to FD and ESD, respectively.

**Key message/Implications**
- In Shanghai household dust, concentrations of PAEs were the highest with CPs as the second most abundant. The high abundance and frequency found for these chemicals are of concern.
- The carcinogenic risk of the phosphate ester, TCEP, for toddlers was only one order of magnitude lower than the threshold value of 10^-6. Aggregate exposure of TCEP through all exposure pathways for toddlers need further attention but is troublesome and will require further action.
- Residents in this study were exposed to low doses of BFRs via house dust.

**Publications from the subproject**

**Researchers involved in the project**
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**References**
Chapter 4:
Scientific output and basis of Chemstrres
Chemstrres has produced a large number of peer reviewed scientific publications, primarily in open access form (see 4.1). The basis for the project is the engaged scientists and personnel that have worked within the project and (4.2). Likewise, the Reference group has made significant contributions to Chemstrres (4.3).

### 4.1 Publications and Doctoral thesis from Chemstrres (alphabetical order)


Chapter 4: Scientific output and basis of Chemstrres

**Thesis for doctoral degree from Chemstrres**


The project involves a large number of professionals from China and Sweden as presented below. The authors of the individual chapters in this book are named without affiliations in the chapters.

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