Dammed and Damned?

Ecotoxicological Impacts on the Yangtze Three Gorges Reservoir, China

Von der Fakultät für Mathematik, Informatik und Naturwissenschaften der RWTH Aachen University zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften genehmigte Dissertation

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Abstract

The Yangtze River is the longest river in Asia and has significantly defined China’s development, due to its rich natural resources. The river’s basin is home to about one third of the country’s population and accounts for the majority of the country’s freshwater resources and fishery production - its fish fauna being one of the richest worldwide. Known as the “Golden Channel”, it is the major artery of China’s inland water transportation system while its shores provide a dwelling for a multitude of industries. These factors define the Yangtze River as a driving force for the country’s economy. These benefits further rose after the Three Gorges Dam began operating at full capacity in 2009, providing flood control and energy production, in addition to improved river navigation of the Upper Yangtze Reaches.

Unfortunately, benefits come with risks. The anthropogenic disturbances caused large concern regarding the environmental impacts on the river basin, which may threaten its valuable resources. One major concern is the threat of water pollution. The Yangtze River was rated among the top 10 rivers at risk in the world, inter alia due to huge amounts of urban sewage, industrial wastewater, agricultural effluents and ship navigation wastes discharged along its course, which are considered to be contributing to a serious fish population decline in the river. Moreover, the construction of the Three Gorges Dam was heavily criticized due to resulting environmental risks growing from the inundation of cities and industrial sites, progressive urbanization and industrialization of the area, as well as rising ship traffic. These risks trigger new pollution scenarios that have the potential to threaten the recently established Three Gorges Reservoir (TGR) ecosystem and the people that depend on it; however, limited information on the actual contamination of the TGR and its impending impact is currently available. Thus, monitoring strategies are demanded in order to initiate and evaluate appropriate control mechanisms and management strategies to protect the vulnerable TGR ecosystem. Consequently, it must be asked: “Is the Yangtze Three Gorges Reservoir not only dammed, but also damned?”. This study comprises three parts. The first section aims at a general comprehensive view on the ecotoxicological status of the Yangtze River, from its origin in the Qinghai-Tibetan Plateau in the west, to its estuary at the East China Sea in the east. In accordance with this, the research on organic pollution along its course and several connected water bodies published in the past two decades were reviewed according to the “triad approach” as a holistic assessment method. Reported organic pollutant levels in water and sediments, as well as potential effects on wildlife and humans measured in vitro, in vivo and in situ were critically discussed and further research requirements identified.

The second section of this study attempts to answer the initial question and focuses on the impacts of organic pollution on the TGR area. In order to record organic contamination and to find links to ecotoxicological impacts, as well as to serve as a reference for ensuing monitoring, several sites in the TGR area were screened analogous to the review by applying the “triad approach” with additional lines of evidence. Sediments and fish of the benthic species darkbarbel catfish (Pelteobagrus vachellii) were sampled between 2011 and 2013 to identify key pollutants, relevant endpoints and hot-spot sites. (a) Sediment was analyzed for 54 relevant organic compounds based on the European Water Framework Directive and (b) tested in vitro with the Ames fluctuation assay for mutagenicity, as well as (c) the ethoxyresorufin-O-deethylase (EROD) induction assay for arylhydrocarbon receptor (AhR) mediated activity.
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Furthermore, (d) sediment was investigated in vivo with the Fish Embryo Toxicity Test (extractable fraction) and (e) Sediment Contact Assay (bioavailable fraction) with zebrafish (Danio rerio) to both test for embryotoxicity/teratogenicity. In situ studies with P. vachellii comprised (f) the quantification of biliary pollutant metabolites and (g) the micronucleus formation in erythrocytes to assess genotoxic impacts. Additionally, activities of hepatic (h) phase I (EROD) and (i) phase II (glutathione S-transferase) biotransformation enzymes were measured in situ to both determine AhR-mediated activities. Furthermore, histopathological alterations in liver and excretory kidney of P. vachellii were evaluated, inter alia to assess immunotoxic impacts. EROD induction was tested in vitro and in situ to evaluate possible relationships between the activity of sediments and in situ effects.

The ecotoxicological evaluation of the Yangtze River and the TGR were supplemented by the third section, which focuses on the capability of fish immune organs to metabolize polycyclic aromatic hydrocarbons (PAHs), an ubiquitous class of organic pollutants, to better understand their role in fish immunotoxicity. To this end, the immune organs, head kidney and spleen, of the rainbow trout (Oncorhynchus mykiss) were tested for their biotransformation capability of the prototypic PAH benzo[a]pyrene (BaP), and compared to the liver as the main detoxification organ. (a) The induction of enzymatic capacity was measured as EROD activity, (b) the organ profiles of BaP metabolites were analyzed in vivo and (c) the rates of BaP metabolite production were assessed with microsomes in vitro.

As identified in the initial section, the main research along the Yangtze River was done on PAHs, polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polychlorinated dibenzodioxins/-furans (PCDDs/DFs) and emerging pollutants, like polybrominated diphenyl ethers (PBDEs) and perfluorinated compounds (PFCs). In general, the water quality of the Yangtze River met Chinese national standards, as well as those of the European Water Framework Directive; however, guideline values were exceeded for PAHs, PCBs and the emerging pollutants nonylphenol and phthalic acid esters at certain sections of the river. The Wuhan section and the Yangtze Estuary exhibited especially stronger pollution than other sections. Bioassays, predominantly displaying the endpoints mutagenicity and endocrine disruption, indicated a potential health risk in several areas. Aquatic organisms exhibited detectable concentrations of toxic compounds like PCBs, OCPs, PBDEs and PFCs. Genotoxic effects could also be assessed in situ in fish. The absolute pollution mass transfer of the Yangtze River is of severe concern for the environmental quality of its estuary and the East China Sea.

In the second part, which focused solely on the TGR area, only PAHs could be detected in sediments from 2011 (165-1,653 ng/g), emphasizing their role as key pollutants of the area. Their ubiquity was confirmed at the identified hot-spot sites Chongqing (150-433 ng/g) and Kaixian (127-590 ng/g) in 2013. Concentrations were comparable to other major Chinese and German rivers’; however, the immense sediment influx suggests a deposition of 216-636 kg PAH/day (0.2-0.6 mg PAH/m²/day), indicating an ecotoxicological risk. PAH source analysis highlighted the primary impact of combustion sources on the more industrialized upper TGR section, whereas petrogenic sources dominated the mid-low section. Furthermore, sediment extracts from several sites exhibited significant impacts of frameshift promutagens in the Ames fluctuation assay. The sediments induced in the in vitro/in vivo bioassays AhR-mediated activities and embryotoxic/teratogenic effects, particularly on the cardiovascular system. These endpoints could be significantly correlated to each other and respective chemical data; however, particle-bound pollutants showed only low bioavailability. The in situ investigations suggested
Abstract

a rather poor condition of *P. vachellii*, with histopathological alterations in liver and excretory kidney. Significant genotoxic impairments in erythrocytes of *P. vachellii* were detected (Chongqing/Kaixian), demonstrating the relevance of genotoxicity as an important mode of action in the TGR’s fish. In addition, fish from Chongqing city exhibited significant hepatic EROD induction and obvious parasitic infestations. The PAH metabolite 1-hydroxypyrene was detected in the bile of fish from all sites. All endpoints in combination with the chemical data suggest a pivotal role of PAHs in the observed ecotoxicological impacts. PAHs, their derivatives and non-target compounds are considered as main causative agents in the TGR area. With regard to the third part on the PAH metabolization capacity of fish immune organs, similar levels of BaP could be detected in the organs of exposed trout, while EROD induction differed significantly between the organs, with the rates of microsomal metabolite formation displaying the same pattern (liver >> head kidney > spleen). In addition, all organs produced the potentially immunotoxic BaP-7,8-dihydrodiol as the main metabolite. This demonstrates the distribution of PAHs into immune organs of fish and that the organs possess the capability to transform them into potentially immunotoxic metabolites. The global conclusion that can be reached from the data produced from this study infers that the Yangtze River and the TGR area are generally in an acceptable state with regard to organic contamination levels. This can be largely referred to a strong dilution effect, which reduces the ecotoxicological risk but does not eliminate it. Mass balance estimations suggest a high input of organic compounds, while registered *in vitro, in vivo* and *in situ* effects demonstrate hidden toxic potentials. The low bioavailability of particle-bound pollutants can be magnified through remobilization, e.g., during the frequently occurring Yangtze River floods. Moreover, the impact of air pollution on the water bodies appears to be considerable, with PAHs occupying a central role. The ubiquity of PAHs is of concern, as they may induce a broad variety of effects, including mutagenicity, AhR-mediated impacts and immunotoxicity. Generally, it was concluded that alternative to risks growing from long-term low-dose chronic exposure, pollution mainly functions as a catalyst for other health impacts, such as cardiovascular diseases and cancer. High-dose acute toxicity primarily occurs at hot-spot sites in highly industrialized and urbanized areas, which strongly suggest a toxicity reduction evaluation. The applied monitoring approach was well suited to provide a comprehensive insight on the ecotoxicological status of the TGR and to further supply lacking data, in order to support the timely initiation of countermeasures to prevent environmental deterioration, as well as detrimental long-term impacts on both the economy and health of the population.
Zusammenfassung


Der zweite Teil der Studie versucht die anfängliche Frage zu beantworten und konzentriert sich auf die Auswirkung von organischen Schadstoffen auf das Gebiet des Drei-Schluchten-Reservoirs. Um sowohl den Kontaminationszustand mit organischen Schadstoffen zu erfassen, als auch Verbindungen zu ökotoxikologischen Auswirkungen zu finden und als Referenz für weitere Monitoringvorhaben zu dienen, wurden verschiedene Standorte im Gebiet des DSR,
Zusammenfassung


Die ökotoxikologische Bewertung des Yangtze und des DSR wurden durch einen dritten Teil ergänzt. Dieser konzentrierte sich darauf in Fischen die Fähigkeit der Immunorgane zu untersuchen polyzyklische aromatische Kohlenwasserstoffe (PAK), eine ubiquitäre Klasse von organischen Schadstoffen, zu metabolisieren. Dies diente dem Zweck ihre Rolle bei der Immuntoxizität in Fischen besser zu verstehen. Dazu wurden die Immunorgane Kopfniere und Milz der Regenbogenforelle (Oncorhynchus mykiss) auf ihre Biotransformationskapazität hinsichtlich des prototypischen PAKs Benzo[a]pyren (BaP) untersucht, und mit der Leber als Hauptentgiftungsorgan verglichen. (a) Die Induktion der Enzymkapazität wurde als EROD Aktivität gemessen, (b) die organotypischen Profile der BaP Metabolite in vivo analysiert und (c) die Produktionsraten der BaP Metabolite mittels Mikrosomen in vitro bestimmt.

Im ersten Teil der Studie konnte festgestellt werden, dass der Hauptanteil der Forschung entlang des Yangtze folgenden Schadstoffen galt: PAKs, polychlorierten Biphenylen (PCBs), Organochlorpestiziden (OCPs), polychlorierten Dibenzodioxinen/-furanen (PCDDs/PCDFs) und „Emerging Pollutants“, wie polybromierten Diphenylethern (PBDEs) und perfluorierten Chemikalien (PFCs). Des Weiteren entsprach die Wasserqualität des Yangtze generell chinesischen Standards und auch denen der Europäischen Wasserrahmenrichtlinie. Jedoch wurden auch an verschiedenen Standorten die Richtlinien für PAKs, PCBs und den „Emerging Pollutants“ Nonylphenol und Phthalaten überschritten. Insbesondere die Region um Wuhan und das Mündungsgebiet des Yangtze wiesen vergleichsweise starke Belastungen auf. Biotests, die vorrangig die Endpunkte Mutagenität und endokrine Störungen anzeigten, wiesen auf potentielle Gesundheitsrisiken in verschiedenen Regionen hin. Weiterhin konnten in aquatischen Organismen messbare Konzentrationen von toxischen Substanzen, wie PCBs, OCPs, PBDEs und PFCs festgestellt werden, sowie gentoxische Effekte in situ in Fischen. Ganzheitlich betrachtet, stellt der absolute Schadstoffmassentransfer des Yangtze ein
Zusammenfassung

besorgniserregendes Problem für die Umweltqualität des Mündungsgebietes und des Ostchinesischen Meeres dar.


Im Hinblick auf den dritten Teil der Studie, die Metabolisierungskapazität von PAKs in Fischimmunorganen, konnten in den Organen belasteter Forellen ähnliche Konzentrationen von BaP festgestellt werden. Im Gegensatz dazu varierte die EROD Induktion signifikant zwischen den Organen, mit demselben Muster bei den Mikrosomen hinsichtlich der Bildungsraten der Metabolite (Leber >> Kopfniere > Milz). Weiterhin produzierten alle Organe das potenziell immunotoxische BaP-7,8-dihydrodiol als Hauptmetabolit. Dies demonstriert die Verteilung von PAKs zu den Immunorganen von Fischen, und deren Fähigkeit PAKs in potenziell immunotoxische Metabolite umzuwandeln.

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Introduction
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Parts of this chapter have been published in peer-reviewed journals:


Chapter I – Introduction

1 Why we should care

What is the leading cause of death in low- and middle-income countries?

It is neither malnutrition or undernutrition, nor diseases like tuberculosis and HIV/AIDS, despite them all being major causes. The Blacksmith Institute, the Green Cross Switzerland and the Global Alliance on Health and Pollution, which ranks the United Nations Environment Program, the European Commission, the World Bank and the Asian Development Bank in addition to other governments and developmental services among its members, announced a surprising cause: pollution (Blacksmith Institute et al. 2015).

Overall, 56 million people died worldwide in 2012, from which about 9 million people perished in consequence of pollution – most of them children. Out of those 9 million people, 8.4 million were killed in low- and middle-income countries, due to exposure to polluted soil, water and air (both household and ambient). Usually people do not die directly from pollution: it acts as a catalyst that enhances the rates of heart disease, chest infections, cancers, respiratory diseases or diarrhea. It is because of this that the World Health Organization considers pollution a risk factor like obesity and malnutrition (OECD Observer 2007, Blacksmith Institute et al. 2015, Fuller 2015, WHO 2015b,e); however, this is just a piece of the picture. It is estimated that 200 million people suffer from body damage, often permanently, and reduced life expectancy due to exposure to heavy metals, obsolete pesticides and other chemicals. Therefore, toxic pollution takes an enormous toll on health and economy (cf. Chapter IV-1.11) (Blacksmith Institute et al. 2015).

China has made a major leap from a developing country to the brink of a developed country in the past decades, spurred by an enormous economic growth; however, benefits often come with consequences – and these consequences severely damage China’s growth (cf. Chapter IV-1.11). China currently faces several environmental challenges (World Bank 2007, Blacksmith Institute et al. 2015); the same challenges that Europe also had to face and partly still does - with the result of an accumulation of knowledge, techniques and legislations in environmental protection.

“Our economy is global and so are the pollutants it generates.” (Fuller 2015) Pollution is not just a regional problem, restricted to a certain area, but can branch out to neighboring countries and overseas, due to air and water transportation pathways and the exchange of goods on a globalized market. Furthermore, the economic and political implications that root in a degenerated environment cannot be overlooked or ignored as they may exacerbate the situation.
Chapter I – Introduction

Developed countries should therefore give priority to these issues and help to eliminate them before they can reach their own doorstep.

This study presents a Sino-German cooperation with the aim to provide an overview on the ecotoxicological status and connected implications of China’s most essential source of drinking water and major artery of economic development - The Yangtze River (cf. Chapter 1-2). Furthermore, it focuses on the ecotoxicological impacts on the reservoir of the most important Chinese hydraulic project – The Yangtze Three Gorges Dam. The applied techniques, the obtained knowledge and drawn conclusions are intended to support a sustainable management of the newly created Three Gorges Reservoir (TGR) ecosystem, and beyond that, of other water bodies in China and pollution affected developing countries.
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2 The Yangtze River

The People's Republic of China has an area of about 9.6 million square kilometers, making it one of the biggest countries on earth with some of the largest rivers on the planet. The Yangtze River (Changjiang), the longest river in Asia (6,300 km), rises from the Qinghai-Tibetan Plateau in the west and crosses the country through 11 provinces. Usually, the river is divided into three major sections: Upper Yangtze Reaches, Middle Yangtze Reaches and Lower Yangtze Reaches (Fig. I-1). The Upper Yangtze (4,300 km) originates from the Geladandong Mountains down to Yichang, comprising a catchment area of 100,100 km². This section can be divided into Upstream TGR (Qinghai-Tibetan Plateau to Chongqing – 3,640 km) and the TGR (Chongqing to Sandouping/Yichang - 660 km). The Middle Reaches (950 km) stretch down to Hukou (outlet of Poyang Lake) with a catchment area of about 68,100 km² (Bergmann et al. 2011). Downstream Hukou, the final 930 km constitute the lower Yangtze River with a total drainage area of 120,000 km² (Chen et al. 2001) until the Yangtze River eventually empties into the East China Sea at the city of Shanghai. The Yangtze River has over 700 tributaries, with major tributaries such as Jialing River, which enters the mainstream at the city of Chongqing, and Han River, which has its inlet at the city of Wuhan. It also connects with important freshwater lakes like Dongting Lake, Poyang Lake and Tai Lake in the Middle and Lower Reaches (Fig. I-1). These tributaries and interlaced lakes form a complete riverine-lacustrine network together with the Yangtze River (Fu et al. 2003).

Life and prosperity emanate from the Yangtze River since centuries. It drains one-fifth of the land area of China and the river basin is home to 400 million people, one-third of the Chinese population. The river basin accounts for 40% of China’s freshwater resources (National Bureau of Statistics - China 2004, Wong et al. 2007). The Yangtze River’s annual runoff is about 9.5×10¹¹ m³ (mean water discharge: 30,200 m³/s), accounting for 52% of the national total runoff (Zhang 1995, National conditions - China 2003), which makes the river the major artery of China’s inland water transportation and the most important source for drinking water, supplying 186 cities (Greenpeace 2010). The river basin also plays a significant role in fishery. It has been reported to account for about 60% of China’s freshwater fishery production (Liu & Cao 1992, Liu et al. 2005), with a fish fauna being one of the richest world-wide (Fu et al. 2003). It provides habitats for about 387 fish species, among which 146 species are unique to the Yangtze River (Yang et al. 2009a). Due to its rich natural resources the river contributes to China’s economic development to a large extent. Known as the “Golden Channel”, it serves as a key factor in inter-province business navigation and the regional economy. The potential
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benefits even grew after the Three Gorges Dam (TGD) operated at full capacity since 2009, including flood control, energy production and improvement of river navigation (Fu et al. 2010) (cf. Chapter I-3). The river is also one of China’s industrial and agricultural key locations. In 2006, over 10,000 chemical enterprises - about half of the total number in China - were located by the river (Yang et al. 2008). The Yangtze River basin provides more than 70% of the country’s rice and 50% of grain production, accounting for 40% of China’s gross domestic product (GDP) (National Bureau of Statistics - China 2004).

However, benefits always go with consequences. The anthropogenic disturbances of the Yangtze River basin leads to increased soil erosion and growing risks for landslides due to deforestation and the impoundments of reservoirs created by overall 45,000 dams in the Yangtze watershed (Wu et al. 2004, Müller et al. 2008). These dams and the increase of river navigation alter the original ecosystems and pose threats to the local biodiversity (cf. Chapter I-4). Beyond that, the river suffers huge amounts of industrial wastewater, urban sewage discharge, ship navigation and oil containing wastewater discharge from ships. It was rated by the World Wildlife Fund (WWF) among the top 10 rivers in the world at risk (Wong et al. 2007). In the 1990s, the organic pollutants of the Yangtze River were monitored by the Yangtze Valley Water Environment Monitoring Center (YVWEMC). 206 hazardous organic chemicals were detected in waters and 106 in sediments, 17 of them belonging to the priority controlled pollutants of America (Wang & Peng 2002). Some organic compounds, like the so-called persistent organic pollutants (POPs), which resist photolytic, biological and chemical degradation (Ritter et al. 1995), pose great ecotoxicological risks to aquatic ecosystems. Later in 2006, the question “How polluted is the Yangtze River?” was raised by Müller et al. (2008). In an extended sampling campaign they studied the concentrations of major ions, nutrients, trace elements and organic pollutants in the Middle and Lower Reaches of the Yangtze River. The growing fraction of wastewater in the Yangtze River was confirmed, which induces rising levels of nutrients, heavy metals and dissolved organic carbon (DOC) accompanied by an astonishing discharge of 500 to 3,500 kg industrial organic chemicals per day. However, they concluded that the concentrations of many anthropogenic compounds were comparable to other major rivers in the world, because high dilution rates mask the enormous discharge of pollutants. Yet they pointed out that economic growth, population and living standards suggest that the concentrations of important water quality constituents are rising and that especially the Yangtze Estuary might face disastrous effects (Müller et al. 2008).
Fig. I-1. Maps of the (A) Yangtze River (Changjiang) Drainage Basin and (B) the Yangtze Delta region. TGR - Three Gorges Reservoir; TGD - Three Gorges Dam; (1) Jinsha River; (2) Yalong River; (3) Dadu River; (4) Ming River; (5) Jialing River; (6) Han River; (7) Wu River; (8) Dongting Lake; (9) Poyang Lake; (10) Tai Lake.
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3 The Three Gorges Project

Under normal conditions, obtaining water of high quality is not a concern in Western Europe. However, in view of climate change, additional efforts are necessary to assure a permanent supply of high-quality drinking water.

The situation is quite different in China. Due to a growing demand for water in households and industry, more and more people are suffering from water scarcity. The Chinese authorities regard the diversion of water from the southern areas in China to the North by water channels as a promising alternative to mitigate this problem (Subklew et al. 2010). Closely linked to these challenging planning and engineering activities are numerous dam construction projects in the southern part of China. The largest dam in China - and one of the largest worldwide - is the Three Gorges Dam, which dams the Yangtze River over a distance of 663 km between the town of Sandouping, Hubei Province, and the Jiangjin district of Chongqing Municipality, to create the TGR.

Over the past 100 years, the annual water discharge of the upper Yangtze at Yichang (near Sandouping) has varied between 5,000 m³/s in January and 40,000 m³/s in the rainy summer months. In the months from June to September, there are also considerable amounts of suspended matter transported. In the course of the last few decades, the periods of both low and high discharges have become more and more extreme so that by the end of the 1970s, the saltwater intrusion from the East China Sea combined with a very low water level had already severely affected the water supply of the city of Shanghai.

The first plans for damming the Yangtze in the region of the Three Gorges and regulating its flow were put forward in 1919, the National People's Congress finally approved the construction plans in 1992 and the dam was completed in 2009. With this gigantic project, the national executive is pursuing the aims of:

- Preventing flooding
- Safeguarding the water supply
- Enhancing navigation
- Generating electric energy

In the future, fluctuations of the water level of up to 30 m will be deliberately applied in the dammed-up section of the river. Up to the start of the rainy period, from the end of May until early June, the water level is lowered to 145 m above sea level (a.s.l.) in order to provide storage
volume for the peak discharge during the following months and to allow sediment flushing. After the maximum water volume has passed, the water level is raised in October to the highest level of 175 m (a.s.l.). Peak energy production then begins. In the months from January to May, the water level is then gradually reduced again to 145 m (a.s.l.) in order to compensate for the lack of precipitation during the dry winter and thus to increase the flow downstream of the dam. This is intended to combat sediment formation in the reservoir in the months from June to September and to wash away some of the existing deposits.

Due to a number of factors the recently established TGR ecosystem faces several environmental challenges.

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**Fig. I-2. Several impressions from the Yangtze River and its tributaries.** View on the Pengxi River Wetland Nature Reserve in Kai County (upper left) and the Yangtze River at Yunyang (upper right), a fisherman in the Pengxi River Wetland Nature Reserve (lower left), the Daning River at Wushan (lower middle) and the Pengxi River near Yunyang (lower right).
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4 Environmental challenges of the Three Gorges Reservoir

Chongqing Municipality (82,403 km²), in which the largest part of the TGR is located, had a population of more than 29 million people in 2011 – 13 million in rural and 16 million in urban areas (National Bureau of Statistics - China 2012). Chongqing city, which incorporates almost half of the urban population (6-7 million) in 2012 (BBC 2012), is already the largest city at the TGR, one of the largest in western China and belongs to the fastest growing cities worldwide. It is expected that another 4 million people will move away from the rural areas to the cities by 2020, with an urbanization rate of 70% (Xinhua 2007b). It has been admitted that overpopulation of the area and pollution, as consequence of the impoundment, pose a serious threat to the vulnerable ecological environment of the reservoir (Xinhua 2007a,b).

As a consequence of the impoundment, already 13 cities, 140 towns and over 1,300 villages have been submerged in addition to more than 1,600 factories and abandoned mines (Smith 2013). They bear the potential to release remaining contamination into the reservoir.

The elevation of the water level improved the navigation of large container ships between Sandouping and Chongqing, and thus became an essential factor for the economic development of this region. The amount of cargo that passed the dam increased by 15.5% per year since the impoundment and reached 106 megatonnes (Mt) per year in 2013 (Xinhua 2014). The increasing traffic, also including passenger shipping, will entail an elevated discharge of contamination into the reservoir.

An increasing number of citizens and progressive industrialization will trigger an increasing amount of wastewater in the urbanized regions, which will challenge the local treatment systems. This means, as the cities grow more domestic and industrial waste ends up in the TGR concentrated especially in these areas. Between 2004 and 2010 about 1,000 Mt urban sewage (53%) and industrial wastewater (47%) were discharged into the TGR area annually during the impoundment (Ministry of Environmental Protection - China 2006-2012). In response the local governments adopted policies on emission reduction for the industry and improved the domestic wastewater treatment capacities from annually 515 Mt in 56 facilities in 2008, to 590 Mt in 71 facilities in 2010. However, both times domestic sewage contributed for about 98%, meaning that still 88 Mt domestic and 548 Mt industrial sewage in 2008, as well as 37 Mt and 307 Mt, respectively, in 2010 were discharged untreated (Ministry of Environmental Protection - China 2010, 2012, Wang et al. 2013).
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Sediment plays an important role in environmental assessment, because it can act as sink and source for pollutants (cf. Chapter I-6) (Ahlf et al. 2002, Hollert et al. 2003, Gerbersdorf et al. 2005, Hilscherova et al. 2007, Wölz et al. 2009). The Yangtze River is one of the largest sediment carriers in the world. Since the operation of the dam annually 151-172 Mt of sediment have been trapped in the TGR (2003-2008) (Yang et al. 2007, Hu et al. 2009a), which accounts for about 60-68% of the sediment entering the TGR from upstream. This has serious consequences for the regions downstream of the dam - especially the Yangtze Estuary -, which rely on continuous sediment supply (Yang et al. 2007, Xu & Milliman 2009).

Another essential factor is the reduction of the river’s flow velocity due to the construction of the dam, which dropped from 2-3 m/sec in average (upper/middle layer) to <0.05-1.5 m/sec (Chen et al. 2005, Wang et al. 2009). This in turn affects the dilution of contamination from point sources, such as urban and industrial wastewater outlets, from where they have been swept away before, as well as the sedimentation rate of suspended particles and adhering contaminants. Thus, it can be expected that the pollution rather accumulates in the area of discharge.

The Yangtze River’s fish fauna is described as one of the richest worldwide, providing plentiful fish resources, but they showed a serious decline with a significantly reduced fishery yield in the past years (Fu et al. 2003, Chen et al. 2009). Changes in age structure and species composition were registered in the catches of the upper Yangtze River section, including the TGR region. The proportion of adult fish decreased, while juvenile and young adults became more abundant. Further, the species composition shifted in its quantitative relationships and decreased in diversity (Chen et al. 2002a,b, 2009). The impoundment of the TGR further threatened endemic and rare fish species, particularly those adaptable to the prior running water conditions, with demersal fishes like the southern sheatfish (Silurus meridionalis), common carp (Cyprinus carpio) and darkbarbel catfish (Peleobagrus vachellii) becoming more important. Yiming and Wilcove (2005) stated the most pervasive threats to Chinese vertebrate animals in general were overexploitation, habitat destruction and pollution, which contributed to the endangerment of 78%, 70% and 20%, respectively, of imperiled species. However, only few quantitative studies on Yangtze fishes are available, but a number of qualitative research demonstrates that aside habitat fragmentation and shrinkage, invasion of exotic species and resources overexploitation, also water pollution is one of main responsible factors for the registered depletion and structural changes of the fish resources (Chen et al. 2009). Industrial and communal sewage discharging toxicants into the environment are considered to trigger the
destruction of spawning grounds, depletion of brood stocks, decrease in production and induction of mortality (Chen et al. 2004).

Chen et al. (2009) warned of the increasing seriousness of pollution in the Yangtze River as consequence of proceeding industrialization and urged for a strengthened environmental monitoring and control of the water pollution to maintain a suitable fishery environment. Moreover, the outlined challenges threaten an important source for drinking water and food for many people. However, little information on contamination and possible impacts is yet available on the region upstream of the dam, particularly of the TGR (cf. Chapter III-1) (Floehr et al. 2013). Thus, monitoring strategies are demanded, in order to initiate appropriate control mechanisms and management strategies of the vulnerable TGR ecosystem.

Fig. I-3. Yangtze Three Gorges Reservoir at Chongqing (left) and sediment residues in the Qingshui brook at Ciqikou, the old town of Chongqing (right). The photo was taken after a flood event in the Jialing River, to which the brook is connected to.
5 The European Water Framework Directive

The “European Water Framework Directive” (EWFD) was adopted by the European Parliament and Council to realize a sustainable management and protection of freshwater resources, with the aim to achieve a good ecological and chemical state of all European lakes, rivers and groundwaters until the year 2015 (EWFD Directive 2000/60/EC). It replaces, merges and renews all parts of the European water protection policy from the 1970s to a consistent, transparent and comprehensive new concept (Hollert et al. 2007). The EWFD comprises several criteria to evaluate the ecological and chemical state of the water bodies according to:

- Biological quality
- Hydromorphological quality
- Physico-chemical quality
- Chemical quality

The biological quality refers to aspects as species compositions and abundance of fish, benthic invertebrate fauna and aquatic flora. The hydromorphological quality focuses on the hydrological regime, river continuity and morphological conditions, while the physico-chemical quality regards to indicators as nutrient concentrations, oxygen balance, salinity, pH, acid neutralizing capacity and temperature. The chemical quality however refers to concentrations of specific synthetic and non-synthetic pollutants in the water (EWFD Directive 2000/60/EC).

“Chemical pollution of surface water presents a threat to the aquatic environment with effects such as acute and chronic toxicity to aquatic organisms, accumulation in the ecosystem and losses of habitats and biodiversity, as well as a threat to human health. As a matter of priority, causes of pollution should be identified and emissions should be dealt with at source, in the most economically and environmentally effective manner.”

(EWFD Directive 2008/105/EC)

In order to meet the “good status”, primarily in terms of chemical quality, several strategies against pollution of water have been initiated (Article 16, EWFD Directive 2000/60/EC). To this end, (i) an number of priority substances were selected and (ii) Member States advised to “adopt measures to eliminate pollution of surface water by the priority substances and progressively to reduce pollution by other substances which would otherwise prevent
States from achieving the objectives for the bodies of surface water” (EWFD Directive 2000/60/EC, European Union 2001a). This first list of compounds was later replaced by a daughter directive, the “Directive on Environmental Quality Standards” (EWFD Directive 2008/105/EC). It added environmental quality standards (EQS) to the list of compounds in surface waters (river, lake, transitional and coastal) and further confirmed them as “priority substances”. Compounds of particular concern were designated as “priority hazardous substances”. As the European Commission is required to repeatedly review the list of compounds a proposal for a new directive (European Union 2012a) was put forward, with the aim to amend the European Water Framework Directive and the Directive on Environmental Quality Standards (European Union 2015a).

A “good chemical status” of a water body is achieved when it meets the EQS for all the priority substances and certain other pollutants listed in Annex I of the Directive on Environmental Quality Standards (Tab. I-1) (Annex V, point 1.4.3 of EWFD Directive 2000/60/EC and Article 1 of EWFD Directive 2008/105/EC).

However, although the “good status” should be achieved for the whole water body the EWFD primarily focused on concentrations of pollutants in the water column. But as suspended particulate matter and sediments also play a crucial role in quality of a water body (cf. Chapter I-6) an inclusion of those compartments into the EWFD was considered necessary (e.g., SedNet 2004, Hollert et al. 2007, Brils 2008). Therefore, the possibility of establishing and applying EQS for sediment as well as for biota, instead of those for water, was included in the Directive on Environmental Quality Standards, whereas “these EQS shall offer at least the same level of protection as the EQS for water set out in Part A of Annex I” (EWFD Directive 2008/105/EC, European Union 2012a).

The European member states were advised to “ensure the establishment of programs for the monitoring of water status in order to establish a coherent and comprehensive overview of water status within each river basin district” (Article 8 of EWFD Directive 2000/60/EC) for all matrices: water, sediment and biota (EWFD Directive 2008/105/EC, European Union 2012a).

These conceptual approaches are well suited to be transferred also to other countries, for example to China.
Table I-1. Priority substances and certain other pollutants according to Annex II of Directive 2008/105/EC (European Union 2015b; last updated 22 April 2015), with proposed priority substances according to the European Union (2012a).

<table>
<thead>
<tr>
<th>No.</th>
<th>Priority substance ( \text{x})</th>
<th>Proposed priority substances ( \text{f})</th>
<th>Certain other pollutants ( \text{k})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alachlor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Anthracene ( \text{x})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Atrazine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Benzene</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Brominated diphenylether ( b,c,x)</td>
<td>Pentabromodiphenylether (congeners 28, 47, 99, 100, 153 and 154)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cadmium and its compounds ( x)</td>
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<td>Chloroalkanes, C10-13 ( b,x)</td>
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<td>Chlorfenvinphos</td>
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<td>Chlorpyrifos</td>
<td>- Chlorpyrifos-ethyl</td>
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<td>1,2-Dichloroethane</td>
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<td>Dichloromethane</td>
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<td>Di(2-ethylhexyl)phthalate ( x)</td>
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<td>13</td>
<td>Diuron</td>
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<td>14</td>
<td>Endosulfan ( x)</td>
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<td>Fluoranthene ( d)</td>
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<td>16</td>
<td>Hexachlorobenzene ( x)</td>
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<td>Hexachlorobutadiene ( x)</td>
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<td>18</td>
<td>Hexachlorocyclohexane ( x)</td>
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<td>19</td>
<td>Isoproturon</td>
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<td>Lead and its compounds</td>
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<td>Mercury and its compounds ( x)</td>
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<td>Nickel and its compounds</td>
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<td>Nonylphenols ( x)</td>
<td>- 4-nonylphenol ( x)</td>
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<td>25</td>
<td>Octylphenols</td>
<td>- 4-(1,1',3,3'-tetramethylbutyl)-phenol</td>
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<td>Pentachlorobenzene ( x)</td>
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<td>Pentachlorophenol</td>
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<td>Polycyclic aromatic hydrocarbons ( x)</td>
<td>- Benzo[a]pyrene ( x)</td>
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<td>- Benzo[b]fluoranthene ( x)</td>
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<td>- Benzo[g,h,i]perylene ( x)</td>
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<td>- Benzo[k]fluoranthene ( x)</td>
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<td>- Indeno[1,2,3-cd]pyrene ( x)</td>
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<td>29</td>
<td>Simazine</td>
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<td>Tributyltin compounds ( a,x)</td>
<td>- Tributyltin-cation ( x)</td>
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<td>Trichlorobenzenes</td>
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<td>Trifluralin ( x)</td>
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<td>Dicofol ( x)</td>
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<td>Perfluoroocane sulfonic acid and its derivatives ( x)</td>
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<td>Quinoxyfen ( x)</td>
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<td>37</td>
<td>Dioxins and dioxin-like compounds ( b,x)</td>
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<td>Aclonifen</td>
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<td>Cybutryne</td>
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<td>Cypermethrin ( h)</td>
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<td>Dichlorvos</td>
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<td>Hexabromocyclododecanes ( l,x)</td>
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<td>Heptachlor and heptachlor epoxide ( x)</td>
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<td>45</td>
<td>Terbutryn</td>
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<td>46</td>
<td>17alpha-ethinylestradiol ( l)</td>
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<td>47</td>
<td>17beta-estradiol ( l)</td>
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<td>48</td>
<td>Diclofenac ( l)</td>
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<td>6a</td>
<td>Carbon-tetrachloride ( l)</td>
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<td>9a</td>
<td>Cyclodiene pesticides</td>
<td>- Aldrin ( l)</td>
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<td>- Dieldrin ( l)</td>
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<td>- Isodrin ( l)</td>
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<td>9b</td>
<td>Dichlorodiphenyltrichloroethane total ( l,m)</td>
<td>D-Dichloro-p-diphenyltrichloroethane ( l)</td>
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<tr>
<td>29a</td>
<td>Tetrachloro-ethylene ( l)</td>
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<td>29b</td>
<td>Trichloro-ethylene ( l)</td>
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Where groups of substances have been selected, typical individual representatives are listed as indicative parameters (with dash, without number); These groups of substances normally include a considerable number of individual compounds. At present, appropriate indicative parameters cannot be given. According to the proposal by the European Union (2012a) only tetra-, penta-, hexa- and heptabromodiphenylether; Fluoranthene is on the list as an indicator of other, more dangerous polycyclic aromatic hydrocarbons; According to the proposal by the European Union (2012a) including tributyltin-cation; These substances have been added in the proposal amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy (European Union 2012a); This includes the following compounds: 7 polychlorinated dibenzo-p-dioxins (PCDDs): 2,3,7,8-T4CDD, 1,2,3,7,8-P5CDD, 1,2,3,4,7,8-H6CDD, 1,2,3,6,7,8-H6CDD, 1,2,3,7,8,9-H6CDD, 1,2,3,4,6,7,8-H7CDD, 1,2,3,4,6,7,8,9-O8CDD. 10 polychlorinated dibenzofurans (PCDFs): 2,3,7,8-T4CDF, 1,2,3,7,8-P5CDF, 2,3,4,7,8-P5CDF, 1,2,3,4,7,8-H6CDF, 1,2,3,6,7,8-H6CDF, 1,2,3,7,8,9-H6CDF, 2,3,4,6,7,8-H6CDF, 1,2,3,4,6,7,8-H7CDF, 1,2,3,4,7,8,9-H7CDF, 1,2,3,4,6,7,8,9-O8CDF. 12 dioxin-like polychlorinated biphenyls (PCB-DL): 3,3',4,4'-T4CB (PCB 77), 3,3',4',5-T4CB (PCB 81), 2,3,3',4,4'-P5CB (PCB 105), 2,3,4,4',5-P5CB (PCB 114), 2,3',4,4',5-P5CB (PCB 118), 2,3',4,4',5-P5CB (PCB 123), 3,3',4,4',5-P5CB (PCB 126), 2,3,3',4,4',5-H6CB (PCB 156), 2,3,3',4,4',5'-H6CB (PCB 157), 2,3',4,4',5,5'-H6CB (PCB 167), 3,3',4,4',5,5'-H6CB (PCB 169), 2,3,3',4,4',5,5'-H7CB (PCB 189); This includes the eight isomers contributing to Cypermethrin, and therefore also alpha-cypermethrin; This includes 1,3,5,7,9,11-Hexabromocyclodecane, 1,2,5,6,9,10-Hexabromocyclododecane, α-Hexabromocyclododecane, β-Hexabromocyclododecane and γ-Hexabromocyclododecane; The inclusion of these substances in Annex X is without prejudice to Regulation (EC) 726/2004 (European Union 2004), Directive 2001/83/EC (European Union 2001c) and Directive 2001/82/EC (European Union 2001b); These eight pollutants, which fall under the scope of Directive 86/280/EEC (European Union 1986) and which are included in List I of the Annex to Directive 76/464/EEC (European Union 1976), are not in the priority substances list. However, environmental quality standards for these substances are included in the Environmental Quality Standards Directive 2008/105/EC. Amended by Directive 88/347/EEC and 90/415/EEC (European Union 1988, 1990, 2015b); This substance is not a priority substance, but one of the other pollutants for which the Environmental Quality Standards are identical to those laid down in the legislation that applied prior to 13 January 2009; Dichlorodiphenyltrichloroethane (DDT) total comprises the sum of the isomers 1,1,1-trichloro-2,2-bis(p-chlorophenylethane, 1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenylethane, 1,1-dichloro-2,2-bis(p-chlorophenylethylene, and 1,1-dichloro-2,2-bis(p-chlorophenylethane; Identified as priority hazardous substance according to EWFD Directive 2008/105/EC and European Union (2012a); Proposed to change status of substance to priority hazardous substance according to European Union (2012a).
6 Role of sediments

Sediments in marine and freshwater systems are complex dynamic matrices, which are composed of organic matter in various stages of decomposition, particulate mineral material with varying size and chemical composition, and inorganic material of biogenic origin, like diatom frustules and calcium carbonate. Many aquatic contaminants are primarily associated with fine deposits that are rich in organic matter, and the way in which these pollutants, particularly semivolatile organic substances, interact with these deposits determines their environmental fate, bioavailability and toxicity (Chen & White 2004). Strong evidence exists that contaminated sediments affect the biocoenoses of invertebrates and cause adverse effects in fish (White et al. 1998, Chen & White 2004, Brinkmann et al. 2013, Hudjetz et al. 2014).

The crucial role of sediments in the quality of water bodies is in particular characterized by their capacity to act as sink and source of pollutants (Ahlf et al. 2002, Hollert et al. 2003, Gerbersdorf et al. 2005, Hilscherova et al. 2007, Wölz et al. 2009). Lipophilic pollutants can adsorb to suspended particulate matter (SPM) and disappear from the water column (“sink”), which reduces their bioavailability. However, as the SPM settles on the ground, the contaminants accumulate in the sediments and have the potential to threaten benthic organisms. In case of incorporated bioaccumulative and persistent compounds they may even biomagnify from here throughout the food chain (Goerke et al. 2004, Gui et al. 2014). Adsorbed particle-bound pollutants can also be remobilized again in consequence of certain events (“secondary source”):

- Bioturbation (Chapman & Power 1992)
- Dumping of sediments (Köthe 2003)
- Storms, currents and floods (Hollert et al. 2000)

Particularly, flood events – as they frequently occur in the Yangtze River – are a major cause for the release of contaminant loads from polluted sediments, which also relocate the remobilized particles and pollutants on inundated river bank soils, which are for example agriculturally used (Wölz et al. 2010, 2011).

A large variety of lipophilic persistent, bioaccumulative and toxic compounds - like organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDE) (cf. Chapter III-1.2) - have a high affinity to adsorb to SPM and accumulate in sediments. Due to their properties members of these compound groups have found their way into several international environmental
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protection legislations, like the “Stockholm Convention on Persistent Organic Pollutants” (UNEP 2001) and the “European Water Framework Directive” (Table I-1) (EWFD Directive 2000/60/EC, EWFD Directive 2008/105/EC, European Union 2012a). The European Member States should take measures to ensure that concentrations of priority substances that tend to accumulate in sediment and/or biota do not significantly increase in these matrices (European Union 2012a). These types of compounds have also been found to play an important role in China (Bao et al. 2012, Lau et al. 2012).

However, the European Sediment Research Network (SedNet) stated that historic contamination of sites due to non-regulated industrialization is a “legacy of the past” that, in combination with remobilization scenarios, threatens the success of the EWFD (SedNet 2004). Thus, due to the risks on human and environmental health originating from organic pollution - current and from the past -, sediment assessment is not only of major importance to industrialized countries. It is of particular importance to developing and newly-industrialized countries that have not yet implemented as strict environmental legislations. Therefore, as demanded by the EWFD the precautionary principle in combination with continuous monitoring of the “whole water body” - including water, sediment and biota - is urgently required (EWFD Directive 2000/60/EC, SedNet 2004, Hollert et al. 2007, Brils 2008, EWFD Directive 2008/105/EC).

Still, a sole monitoring of chemical concentrations in water and sediment with chemical-analytical methods is insufficient. That way only selected compounds are in the scope of the quality assessment, and an even larger variety of toxic non-target parent compounds and metabolites is not considered. Moreover, aspects like bioavailability of the toxic compounds, their synergistic and antagonistic effects, as well as the metabolism in the organisms is not taken into account. Thus, in order to acknowledge the complex situation in the field a holistic assessment in environmental monitoring is demanded, that considers not only the concentrations of selected compounds, but integrates them with the toxic effects the environmental sample may induce (Heugens et al. 2001, SedNet 2004, Hollert et al. 2007, Brils 2008, Hollert et al. 2009, Malaj et al. 2014, Wernersson et al. 2015).

Fig. I-4. Sediment with organic material.
Photo by Peggy Heine.
7 The triad approach

One approved holistic assessment method is the triad approach by Chapman (1990) (Fig. I-5). It integrates several lines of evidence (chemical analysis, *in vitro/in vivo* bioassays and *in situ* biomarkers) to a comprehensive evaluation. While chemical analysis allows to assess the hazard originating from the environmental sample based on the detected compounds, do bioassays and field inventory aim at relevant ecotoxicological endpoints, that assess the risk originating from the sample and the impact on the ecosystem, respectively, and thereby complement the picture (Chapman 1990, SedNet 2004, Chapman & Hollert 2006). By comparison of the individual parts links can be drawn to determine the proportion of the identified compounds on the detected toxicities, the responsible toxicity pathways and the relevance of laboratory screenings compared to the situation in the investigated ecosystem.

![Chemical Analyses](image1)

**Fig. I-5. Conceptual model of the triad approach with additional lines of evidence.** According to Chapman (1990) and Chapman and Hollert (2006). An integrated approach to evaluate the ecotoxicity of water, sediment, soil and suspended particulate matter, as well as state of contamination in local fish, in combination with chemical analysis of the compartments.
8 Relevant ecotoxicological endpoints

A number of relevant ecotoxicological endpoints exist that can be evaluated with a number of bioassays and biomarkers (Wernersson et al. 2015). As outlined before is the catalytic function of pollution to enhance the rates of, e.g., cancer and heart diseases, of particular concern (Blacksmith Institute et al. 2015, Fuller 2015). As reviewed by Floehr et al. (2013) (cf. Chapter III-1) a large variety of organic pollutants, particularly aryl hydrocarbon receptor agonists and dioxin-like compounds, could be measured in the Yangtze River, which rise great concern as they possess the potential to induce lethality, carcinogenesis, tumor promotion, cardiovascular disorders, immunotoxicity, embryotoxicity, teratogenicity and hepatotoxicity in a variety of species already in low concentrations (Ahlborg et al. 1992, Peterson et al. 1993, Cantrell et al. 1998, Hilscherova et al. 2000, Incardona et al. 2004, Sundberg et al. 2005, Teraoka et al. 2010, Brette et al. 2014). Therefore, in the following, endpoints of specific interest shall be described.

8.1 Mutagenicity and genotoxicity

Mutagenicity describes permanent changes in the structure and/or amount of genetic material of the organism, which can lead to heritable changes in its function. It includes gene mutations as well as structural and numerical chromosome alterations (Eastmond et al. 2009). Further, genotoxicity is a broader term, because it includes all directly and indirectly mediated adverse effects on genetic information, like damage to DNA and/or cellular components regulating the fidelity of the genome. It refers to the capacity to give rise to mutations, but do not necessarily become evident as them (Eastmond et al. 2009, UKCOM 2011).

Due to their impact on the genetic material of the organism, mutagenic and genotoxic effects on cellular level can have impairments on the organism itself – like the formation of cancer -, but also multigenerational impacts – by alteration of the genome -, thus effecting the population and ecosystem level (White et al. 1999, Diekmann et al. 2004). Sediments that are contaminated with mutagenic substances pose a hazard to indigenous biota (Chen & White 2004). It has been shown that fish populations in rivers and lakes in industrial areas in the USA and Europe displayed an elevated prevalence for tumors (Balch et al. 1995). As a possible result of genotoxic effects on parental cells the formation of micronuclei can be observed in the daughter cells after cell division (Fig. 1-6) (Leme & Marin-Morales 2009). For convenient application, blood has been proven to be a sensitive compartment for genotoxicity measurement in fish, which can be evaluated with the in situ micronucleus assay (cf. Chapter II-2.7.2) (Kilemade

![Fig. I-6. Micrograph of an erythrocyte with micronucleus (arrow). Nuclei and micronuclei were stained using acridine orange dye.](image)

### 8.2 Dioxin-like toxicity and the Ah receptor

The aryl hydrocarbon receptor (AhR) is a transcription factor located in the cytosol, whose activation depends on binding of ligands (Hilscherova et al. 2000). The AhR plays a central and complex role in the regulation of basic biological processes, like cell cycle progression, cellular differentiation, apoptosis and tumorigenesis (Marlowe & Puga 2005, Hahn et al. 2009, Ma et al. 2009). However, it has also been shown that the receptor is involved in the mediation of toxic effects (Hankinson 1995, Bittner et al. 2006, Olsman et al. 2007). For example, it is proposed that a hyper-activation of the AhR leads to a misregulation of target genes integrated in these basic processes, further resulting in the manifestation of toxic impacts (Carney et al. 2004). The strength with which the ligands bind is directly proportional to the toxicity, transcriptional activity and the AhR-mediated enzyme activity (Safe 1995). The AhR mechanism is shown in Figure I-7.
Fig. I-7. **The AhR pathway and mechanism of AhR-mediated response in cell bioassays.** Adapted and modified from Hilscherova et al. (2000). The cytosolic AhR in its inactive state is bound to two heatshock proteins (hsp 90). After binding of a ligand to the cytosolic AhR, the hsp 90 proteins dissociate from the complex and the receptor ligand complex is activated. The complex is then translocated into the nucleus, where it forms a dimer with the Ah receptor nuclear translocator (ARNT) protein. As a result, these elements form a transcription factor, which binds with high affinity to specific dioxin/xenobiotic-responsive elements (DRE/XRE) on the DNA. This regulates the transcription of several genes to mRNA, which is translated to the respective gene products – like phase I (e.g., CYP450) and phase II (e.g., GST) biotransformation enzymes (Denison & Heath-Pagliuso 1998, Hankinson 1995, Hilscherova 2000). In order to assess the toxic potency of single substances and mixtures of AhR inducers the activity of the regulated enzymes (e.g., CYP1A ethoxyresorufin-O-deethylase – EROD) can be measured directly in vitro, in vivo and in situ by their conversion rate of a pigment (e.g., 7-ethoxyresorufin to resorufin) (Behrens & Segner 2005, Eichbaum et al. 2014, Schiwy et al. 2014). Alternatively, in transgenic cell lines that carry a luciferase reporter gene the relative emission of light can be quantified (Murk et al. 1996, Sanderson et al. 1996).

The ability of the ligands to bind the AhR strongly depends on their shape. Typical substances are hydrophobic aromatic compounds with a planar structure. Either the complete molecule is of particular size and planar structure or only a part of it, which fits the binding sites (Poland & Knutson 1982, Lewis et al. 1986, Hilscherova et al. 2000). The formerly strongest known AhR inducer was 2,3,7,8-tetrachlorodibenzo-p-dioxin, a polychlorinated dibenzodioxin (PCDD). Compounds with a similar planar structure and which are able to elicit toxic effects similar to TCDD are therefore called dioxin-like compounds. Members of this category are along PCDDs, also congeners of PAHs, PCBs, polychlorinated dibenzofurans (PCDFs) among many others.
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(Hilscherova et al. 2000, for review Eichbaum et al. 2014). Dioxin-like compounds rise great concern as they possess the potential to induce lethality, carcinogenesis, tumor promotion, immunotoxicity, embryotoxicity, teratogenicity and hepatotoxicity in variety of species already in low concentrations (Ahlborg et al. 1992, Peterson et al. 1993, Hilscherova et al. 2000). Most of these effects, caused by dioxin-like compounds, are mediated via the Ah receptor (Hankinson 1995, Bittner et al. 2006, Olsman et al. 2007).

Further, dioxin-like compounds are also known inducers of developmental disorders that aim at the cardiovascular system, among them edema of the pericardium (Cantrell et al. 1998, Sundberg et al. 2005) and decreased blood circulation (Cantrell et al. 1998, Teraoka et al. 2010) in fish. Incardona et al. (2004) described cardiac dysfunction as part of a characteristic suite of abnormalities after exposure to complex PAH mixtures from petrogenic sources. They further highlighted secondary consequences of defects in the cardiac function on later stages of cardiac, renal, nervous and craniofacial morphology. Furthermore, Brette et al. (2014) demonstrated a cardiotoxic mechanism, by which water accommodated fractions of PAHs containing crude oil had a serious impact on the regulation of cellular excitability via direct effects on ion channels, with consequences for life-threatening arrhythmias in fish and other vertebrates.

The Ah receptor is also involved in the detoxification and excretion systems of organisms. After the uptake of xenobiotics via various pathways - e.g., the lungs, gills, skin or the gastrointestinal tract - they are distributed via the blood system. Hydrophilic compounds are dissolved directly in the serum and hydrophobic compounds adsorbed to macromolecules, like albumin, globulins and lipoproteins (Calabrese 1990, Baker 2002). Hydrophilic xenobiotics can be easily excreted by the respective pathways, e.g., via urine. Hydrophobic xenobiotics however have to be metabolized to water soluble compounds before they can be excreted. The respective detoxification and excretion systems in vertebrates are located in several organs, but specifically in the hepatocytes of the liver (Stegeman & Hahn 1994). These processes are part of the oxidative metabolism of endogenous substances, like fatty acids and steroids (Volotinen et al. 2009), and basically consist of three phases (Fig. I-8):

- Metabolization (phase I)
- Detoxification (phase II)
- Excretion (phase III)
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Fig. I-8. Biotransformation process of a lipophilic xenobiotic. Adapted from Fent (2013).

The biotransformation of lipophilic xenobiotics in phase I is catalyzed by enzymes that oxidate, hydrolyse or reduce the substances in order to generate metabolites with hydroxyl groups. The enzymes in phase II conjugate the hydroxylised metabolites to endogenous molecules, like glutathione, sulfate or glucuronic acid to increase the water solubility. In phase III are the compounds excreted via urine, or via bile in case of high molecular substances (Benson & Di Giulio 1992, Fent 2013). However, it may also happen that in this progress non-toxic compounds are bioactivated. For example, several higher molecular PAHs gain mutagenic properties after their metabolic activation to electrophilic diol epoxides in phase I (Penning et al. 1999). Furthermore, persistent lipophilic compounds that can neither be detoxified nor excreted may bioaccumulate in the adipose tissue of the organisms, from which they can be slowly released into the bloodstream again or remobilized in higher concentrations when the adipose tissue is degraded, e.g., in case of weight loss or lactation (LaKind et al. 2009, for review La Merrill et al. 2013).

The Ah receptor regulates some of the enzymes involved in phase I and phase II biotransformation processes and their activity can be utilized to measure AhR mediated dioxin-like activity and as an indicator of exposure to dioxin-like compounds.

A prominent class of phase I biotransformation enzymes are cytochrome P450 dependent monoxygenase (CYP), that catalyze the oxidation and reduction of xenobiotics. The ethoxyresorufin-O-deethylase (EROD) belongs to the CYP1A subfamily and is activated by the AhR, e.g., after binding of PAHs, PCBs or PCDDs/PCDFs (for review Van der Oost et al. 2003). The EROD activity can be measured via in vitro bioassays - like the EROD induction assay with rainbow trout liver cells - to exemplarily determine the AhR-mediated dioxin-like activity of environmental samples – like water and sediment – (cf. Chapter II-2.6.2) (for
review Eichbaum et al. 2014), as well as in organisms as in vivo and in situ biomarker (cf. Chapter II-2.7.3) (Hilscherova et al. 2000, Whyte et al. 2000, Behrens & Segner 2005, Brack et al. 2005, Hallare et al. 2011, Brinkmann et al. 2013, Schiwy et al. 2014). Further, a prominent representative of phase II biotransformation enzymes is the glutathione S-transferase (GST). It catalyzes the conjugation of glutathione to xenobiotics (Dietrich & Kaina 2010), is also mediated inter alia by the AhR and can be utilized as a biomarker as well (cf. Chapter 2.7.3) (Hayes & Pulford 1995, Sen & Kirikkalan 2004, Schlenk et al. 2008, Kammann et al. 2014). Both groups play an important role in organisms in the detoxification and excretion of xenobiotics (Reed 1990, Sen & Semiz 2007, Lopez-Galindo et al. 2010).

8.3 Embryotoxicity and teratogenicity

Invertebrates in early life stages are in a particular sensitive state and more susceptible to toxicants than adult organisms (Herkovits et al. 1997, Hutchinson et al. 1998). Embryotoxicity describes the induction of effects on an embryo that result in abnormal development or death. Teratogenicity refers to the capability to cause malformations or defects to an embryo or foetus. In order to test the embryotoxic and teratogenic effects on fish the in vivo Fish Embryo Toxicity Test (FET) (cf. Chapter II-2.6.4) (Braunbeck et al. 2005) and Sediment Contact Assay (SCA) with eggs of Danio rerio (cf. Chapter II-2.6.5) (Hollert et al. 2003) have been proven to be suitable methods in ecotoxicological assessment to determine the toxicity of the extractable (FET) and bioavailable (SCA) fraction of water and sediment. In particular, as the FET can serve as a substitute for the acute toxicity test with adult fish (OECD guideline 203) registering mortality, it also allows to record sublethal impacts that might affect the overall fitness of the fish (Nagel 2002, Braunbeck et al. 2005, Lammer et al. 2009).

8.4 Immunotoxicity

Immunity is an ecologically relevant trait, which is crucial for the survival of individual organisms as well as the growth of populations against the pressure of pathogens in their environment (Segner et al. 2012). Many pollutants possess immunotoxic properties that modulate immune parameters, for example those of fish (Hoeger et al. 2005, Reynaud & Deschaux 2006, Casanova-Nakayama et al. 2011, Segner et al. 2012).

In teleost fish, a major immune organ is the anterior portion of the kidney (head kidney). This organ is a primary site of hematopoiesis in teleost, containing both myeloid and lymphoid leukocytes as well as endocrine cells (Powell 2000, Carlson & Zelikoff 2008). A second
important immune organ of teleost fish is the spleen, which is believed to function similar to the mammalian spleen (Carlson & Zelikoff 2008). The immune cells in these organs are closely associated with the blood system, partly acting as filtering system for the circulatory system, and therefore they are highly accessible to toxicants. In fact, a wide variety of chemicals has been reported to compromise immune functions of fish and to modulate susceptibility to infectious pathogens (Rice 2001, Carlson & Zelikoff 2008, Segner et al. 2013). One class of chemicals that has been repeatedly shown to affect immune functions of fish are PAHs (Arkoosh et al. 2001, Carlson et al. 2002, Hutchinson et al. 2003, Carlson et al. 2004b, Reynaud et al. 2004, Kennedy & Farrell 2008, Danion et al. 2011).

Many of the toxic effects of PAHs such as mutagenicity and cancerogenicity are not caused by the parent compounds but by the metabolites. PAH biotransformation is initiated by CYPs (Xue & Warshawsky 2005). The monooxygenases convert PAH parent compounds into dihydrodiols, phenolics and epoxide intermediates, which then are further metabolized and eventually excreted (Shimada 2006). The question is whether PAH metabolites are also responsible for the immunotoxic effects of PAHs. For mammals, there exists indeed strong evidence that PAH immunotoxicity is not caused by the parent compounds but by their metabolites. Although the main site for PAH metabolism is the liver, the immunotoxic metabolites appear to be generated directly in the immune cells, i.e. by in situ biotransformation (Burchiel & Luster 2001, Ioannides & Lewis 2004, Yusuf et al. 2007). Also for teleost fish, it has been suggested that in situ metabolism of PAHs is causatively involved in PAH immunotoxicity. Carlson et al. (2004a,b) showed that in vivo treatment of medaka (Oryzias latipes) with the prototypic PAH, benzo[a]pyrene (BaP), resulted in immunotoxic effects, which could be ameliorated by co-treatment with alpha-naphthoflavone (ANF), an inhibitor of CYP1A activity. While this result might still be explained by metabolism of BaP in the liver and subsequent transport of the metabolites into the immune organs, the finding of Carlson et al. (2004b) that ANF ameliorated BaP immunotoxicity also in isolated medaka immune cells points to an involvement of PAH metabolism directly in the immune cells. As concluded by Carlson et al. (2004b), “results of this study suggest that (i) BaP-induced suppression of medaka humoral immunity relies upon the CYP1A-catalyzed production of immunotoxic BaP metabolites and (ii) BaP metabolites may be created in situ, directly by specific cells within the kidney lymphoid tissue”. What is lacking so far is the final piece in the picture, that is the demonstration that fish immune organs are capable of in situ PAH metabolism.
9 The darkbarbel catfish (*Pelteobagrus vachellii*)

The darkbarbel catfish (*Pelteobagrus vachellii*, Richardson 1846) (Fig. I-9) is a bottom-dwelling bagrid catfish that can be found primarily in freshwater. It is a widespread species, regionally distributed over China, Korea and Vietnam and has also appeared in Hong Kong as invasive species. In China, *P. vachellii* can be found in the Yellow River in the north, the Yangtze River in the center, as well as in Guangdong Province, Hainan, in the south of the country (Chan & Ho 2011).

*P. vachellii* is signified by an elongated and scaleless body, with a round anterior part, a laterally compressed posterior part, and a dorso-ventrally compressed head with large eyes and four pairs of slender barbels. The nasal barbels reach over the eye and the outer mandibular barbels extend to the base of the pectoral fin spine. The maxillary barbels reach over the gill openings and end behind the base of the pectoral fin spines. The pectoral fin spine possesses a sharply pointed tip and a serrated posterior edge. The dorsal fin has two spines. The first is very short, smooth and covered by skin. The second is long with a serrated posterior edge. The adipose fin is located slightly beyond the midpoint of the dorsal fin and caudal fin and has a short base. The caudal fin is deeply forked. The body is generally colored in a grayish-brown with a pale yellow abdomen, greyish-black edges at all fins and a complete lateral line. The maximum body length is determined with about 300 mm (Chan & Ho 2011). The common length of *P. vachellii* is given with 210 mm (Nichols 1943).

As a benthic species it feeds mainly on worms, small fishes and invertebrates. It shows nocturnal activity, with a low to medium migration (Chen et al. 2002c, Chan & Ho 2011). The typical activity level of bagrid catfish has been reported to be sluggish (FishBase 2012). The darkbarbel catfish is harvested as wild fish and raised for food in aquacultures, thus possessing a major economic importance (Ministry of Environmental Protection - China 2007, Zeng et al. 2010, Chan & Ho 2011).

![Fig. I-9. Darkbarbel catfish (*Pelteobagrus vachellii*).](image)
Chapter I – Introduction

10 The Sino-German Yangtze Project

Water of good quality is one of the basic needs of human life. Worldwide, great efforts are being undertaken for an assured water supply. In this respect, one of the largest water technology projects worldwide is the Yangtze Three Gorges Dam. There is a need for extensive scientific and technical understanding of the challenges arising from this large hydrological engineering project. German and Chinese groups from various scientific fields are collaborating to provide knowledge for the sustainable management of the reservoir within the Sino-German joint research project “Sustainable management of the newly created ecosystem at the Three Gorges Dam” (Yangtze Project) (Bergmann et al. 2011).

As there are many technical, ecological, and social challenges linked with such a large project, the German Federal Ministry of Education and Research (BMBF) has been providing financial support for five German research institutions to perform applied research on changing land use, soil erosion, mass movements, and matter fluxes in this highly dynamic ecosystem since 2008/2009. Between August 2010 and July 2014, another six German partners received funding for research on sustainable water management (Yangtze-Hydro). Within Yangtze-Hydro, they cooperated with Chinese research groups from a number of universities, institutes of the Chinese Academy of Sciences, the China Research Academy of Environmental Sciences, and other research centers. Financial support was given by the Chinese Ministry of Education and the Ministry of Science and Technology. While the German side was coordinated by the Research Centre Jülich, the Chinese side operated under the leadership of the governmental State Council Three Gorges Project Construction Committee and the Tongji University, Shanghai (Küppers et al. 2011). In this chapter, the objectives of the joint project are introduced.

The Yangtze-Hydro Project is related to the most relevant changes in water and sediment quality as:

- Eutrophication by wastewater and agrochemicals, which may lead to algae blooming and water hyacinth growth
- Re-solution of pollutants from flooded urban, industrial and agricultural areas
- Sediment accumulation along the reservoir and especially in front of the dam
- Possibly unknown contamination of toxic inorganic and organic trace compounds from industry, municipal wastewater discharge, landfill deposits, and waste

In order to understand the contamination situation in the reservoir, the underlying processes and to offer potential solutions the German Consortium consisted of four work packages:
Chapter I – Introduction

- Dynamics of physico-chemical parameters within the new reservoir
- Survey of organic pollutants in water with regard to Chinese and German standards for water quality aspects and investigation of suitable water technologies to obtain clean and safe drinking water
- Behavior, transformation and effects of organic pollutants in water, sediment and biota
- Degradation of organic contaminants

The overall aims of the German Yangtze-Hydro projects is to support the Chinese partners in the field of sustainable management of the Yangtze Three Gorges Dam ecological system, where the Chinese partners have already obtained a great deal of experience and have developed a variety of strategies to deal with the upcoming challenges caused by the dam project.

Fig. 1-10. Logo of the Yangtze Project.

10.1 The MICROTOX Project – Ecotoxicology

A large variety of potential pollution input sources exist in the TGR area. The submerged agricultural and industrial areas, as well as point and non-point sources from agriculture, households, traffic and industry may release high amounts of organic and inorganic pollutants and nutrients into the reservoir (Henderson et al. 2007, Moeckel et al. 2008, Yang et al. 2010). Therefore, the behavior and fate of pollutants in the reservoir, as well as the effects on local organisms need to be understood to assess the risks for the TGR ecosystem. Moreover, it is crucial for the local residents that rely on the reservoir area as a source of drinking water and food – for example, from fishery or agricultural sites that are repeatedly submerged due to the reservoirs water fluctuation.

The subproject “Transformation, Bioaccumulation and Toxicity of Organic Micropollutants in the Yangtze Three Gorges Reservoir (MICROTOX)” was conducted at the Institute of
Chapter I – Introduction

Environmental Research, RWTH Aachen University, Germany. It aimed at the integrated assessment of ecological aspects of the TGR, with ecotoxicological impacts and environmental behavior of organic pollutants.

To this end, the subproject consisted of three major modules:

- Fate and behavior of environmental pollutants
- Bioaccumulation and magnification in aquatic food webs
- Ecotoxicity of sediments and analysis of biomarkers of toxic stress

In order to study observable degradation patterns related to the altered fluctuation regimen of the TGR the commonly used rice herbicide Propanil was applied as a model substance. This was realized in a study mimicking the alteration of rice field soil between unsubmerged and submerged over a temporal course. The same herbicide was also used as model substance to investigate the bioaccumulation of parent and metabolized substances in a realistic simulation environment.

The overall aims of the module “Ecotoxicology” were:

- Development and application of a monitoring strategy for future implementation (proof of concept)
- Evaluation of selected areas based on this strategy
- Assess the risk for humans and the ecosystem

The major objective was to support environmental management strategies in China - particular of the TGR - by knowledge transfer, in order to minimize environmental deterioration.

The basic concept of the monitoring strategy was set by the triad approach with additional lines of evidence (cf. Chapter I-7) (Chapman 1990, Chapman & Hollert 2006). To this end, sediment samples and fish were collected at several sites in the TGR and its watershed in the administrative region of Chongqing Municipality. They were analyzed to (i) record the organic contamination and (ii) determine the ecotoxicological effects, as well as (iii) to assess possible links between contamination, biochemical responses and ecologically relevant impacts on fish from the field.

The specific aims and the detailed concept are outlined in Chapter I-11.2 and Chapter II-2.1, respectively.
11 Aims of this study

With regard to the unknown status of ecotoxicological impacts of organic pollution on the
dammed Yangtze Three Gorges Reservoir section is the consequential question “Is the Yangtze
Three Gorges Reservoir not only dammed, but also dammed?”. In order to answer it comprises
this study three parts: (i) It evaluates the ecotoxicological status of the Yangtze River to assess
the general situation, (ii) it investigates the impacts of organic pollution on the river section of
particular interest – the Three Gorges Reservoir –, and (iii) substitutes the previous parts with
an examination of the capability of fish immune organs to metabolize PAHs – an ubiquitous
class of organic pollutants – to better understand their role in fish immunotoxicity.

11.1 The ecotoxicological status of the Yangtze River

It is reasonable to ask whether there is actually a pollution problem in the Yangtze River due to
the high mass transport of water and sediment despite all contamination sources. Is the pollution
problem solved by simple dilution? Is the Yangtze River capable to dilute pollutant levels to a
non-toxic degree, without considerable consequences for wildlife and humans? To answer this
question, on the one hand data about pollutant levels are required, on the other hand it should
be considered to acquire knowledge about possible effects which might be induced by these
(even low) concentrations. Even smallest concentrations of certain contaminants are capable to
induce toxic effects, and bioaccumulation as well as chronic toxicity is not negligible. In order
to complement the work of Müller et al. (2008), this chapter summarizes the current state of
knowledge, comprising published data of the last two decades, regarding ecotoxicological
investigations on organic pollutants in the Yangtze River.

The information was divided into three major chapters according to the conceptual strategy of
the triad approach (cf. Chapter I-7) (Chapman 1990) (Fig. I-5).

- Concentrations of organic pollutants, including POPs, like PAHs, PCBs, OCPs and
  other hazardous substances, in water and sediments were critically reviewed.
- A series of screening bioassays were referred to, inter alia with the endpoints
genotoxicity, mutagenicity and estrogenic activity.
- Available in situ studies were reviewed according to pollutant levels and adverse effects
  in aquatic organisms, like fish, mollusks and crabs.

The main objective was to link chemical pollution to ecotoxicological effects, in order to
provide a clear and comprehensive view on the pollution status of the Yangtze River.
11.2 Ecotoxicological impacts on the Three Gorges Reservoir

The recently established TGR is a section of the Yangtze River of particular interest. It is a source of food and water for millions of people, and the Three Gorges Dam possesses a high economically, social and political relevance. It has been admitted that overpopulation of the TGR area and pollution, as consequence of the impoundment, pose a serious threat to its vulnerable ecological environment (Xinhua 2007a,b). However, only little information on the status of organic pollution and ecotoxicological impacts on the TGR are available (cf. Chapter III-1) (Floehr et al. 2013). Thus, it is of crucial importance to evaluate the situation, as well as to develop and initiate comprehensive monitoring programs, which integrate the concentrations of chemicals with the toxicity of environmental compartments and impacts on biota, in order to prevent environmental degradation in the long-term.

The overall aims were:

- Development and application of a monitoring strategy for future implementation (proof of concept)
- Evaluation of selected areas based on this strategy
- Assess the risk for humans and the ecosystem

The main objective was to support environmental management strategies in China - particular of the TGR - by knowledge transfer, in order to minimize environmental deterioration.

The basic concept of the monitoring strategy was set by the triad approach with additional lines of evidence (cf. Chapter I-7) (Chapman 1990, Chapman & Hollert 2006). The detailed concept and procedure is described in Chapter II-2.1.

To this end, the specific aims were to:

- Identify relevant organic compounds to be measured in the sediment
- Identify relevant ecotoxicological endpoints for in vitro, in vivo and in situ measurement
- Identify a suitable monitoring fish species
- Triad A: Chemical analysis
  
  Record the organic pollution status:

  - Of 54 organic chemicals based on the EWFD
Chapter I – Introduction

- **Triad B: In vitro/in vivo bioassays**
  Determine ecotoxicological effects of sediments *in vitro/in vivo*:
  - Mutagenicity, EROD induction (phase I biotransformation), embryotoxicity/teratogenicity

- **Triad C: In situ biomarkers**
  Assess impacts on the monitoring fish species *in situ*:
  - Genotoxicity, EROD induction (phase I biotransformation), GST induction (phase II biotransformation), histopathological alterations, biliary PAH metabolites

- Discuss the results in relation to each other and in context of water data obtained by the project partners

This should enable to:

- Evaluate the ecotoxicological relevance of potentially contaminated sediment
- Determine in what way is the local fauna, e.g., fish species, affected
- Assess possible links between biochemical responses and ecologically relevant impacts on fish from the field, by assigning possible responsible toxicity pathways
- Identify responsible priority pollutants
- Assess the proportion of the identified compounds on the detected toxicities
- Deduce possible contamination sources
- Identify hot-spot areas
- Evaluate the impact of the tributaries - as dischargers of pollution - on the mainstream
- Evaluate the relevance of laboratory screenings compared to the situation in the investigated ecosystem
11.3 PAH metabolism in immune organs

It has been suggested that the production of PAH metabolites in the immune cells is a mechanism through which PAHs cause immunotoxicity, but it had not been demonstrated yet whether immune organs indeed are capable of PAH metabolism.

Thus, the main objective was to clarify the capability of fish immune organs for \textit{in situ} metabolism of BaP.

More specifically, it investigates:

- Patterns and relative levels of BaP metabolites in fish immune organs, both in the intact fish and in microsomal preparations \textit{in vitro}
- The rates at which BaP metabolites are produced
- How rates and patterns change after exposure of the fish to BaP

The fish model used for this purpose was rainbow trout (\textit{Oncorhynchus mykiss}). The immune organs examined included head kidney and spleen, with the liver used as benchmark.
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12 Funding

The first two parts were conducted in close cooperation with the Tongji University, Shanghai, and the Chongqing University, Chongqing, as part of the “MICROTOX” project (“Transformation, Bioaccumulation and Toxicity of Organic Micropollutants in the Yangtze Three Gorges Reservoir”; Project reference: FKZ 02WT1141). It is integrated into the sino-german joint environmental research program “Yangtze-Hydro - Sustainable Management of the Newly Created Ecosystem at the Three Gorges Dam” (Bergmann et al. 2011; www.yangtze-project.de), as part of the research cluster “Pollutants/Water/Sediment - Impacts of Transformation and Transportation Processes on the Yangtze Water Quality”. The project has been financed by the Federal Ministry of Education and Research, Germany (BMBF), and has been further supported by a cooperation project with Chinese colleagues sponsored by the BMBF DLR and the Chinese 111 Program. The third part was financially supported by the European Commission through the Marie Curie Initial Training Network “KEYBIOEFFECTS” (“Cause-effect relationships of key pollutants on the European rivers biodiversity”; Project reference: 35695). The funding sources had no involvement in the study.
Chapter II

Materials & Methods
-Part 1-

The ecotoxicological status of the Yangtze River

This chapter has been published as part of an article in a peer-reviewed journal:

Chapter II – Materials & Methods

1 The ecotoxicological status of the Yangtze River

1.1 Literature research

The literature research was carried out based on the triad approach (Chapman 1990, Chapman & Hollert 2006) (Fig. II-1), which combines exposure (chemical analyses) and effect analyses (acute and mechanism-specific toxicity) with in situ biological assessment that can be used to assess effects on organisms in the field (Chapter I-7). The data used for this literature review (Chapter III-1) were obtained from a broad variety of platforms such as Web of Knowledge, PubMed, Google scholar, Wanfang and China National Knowledge Infrastructure (CNKI). Some of the papers were published in Chinese. The time frame was set to browse the recent literature of the last two decades (1990-2012). The chosen keywords included Yangtze River/Changjiang (Fig. II-2) and Yangtze River/Changjiang in combination with …sediment, …water, …toxicity, …ecotoxicity, …biotest, …organic pollutants or …persistent organic pollutants. Overall, the number of scientific publications on the Yangtze River has grown significantly during the past years (source: Web of Science, searching for Yangtze River/Changjiang, in article’s topic) (Fig. II-2). In total 84 articles fit into the conceptual approach of this review and have been evaluated. The predominant research on the ecotoxicological status of the river focuses on chemical concentrations in the compartments water and sediment. Research applying bioassays in this area has just started up and only limited literature was found. Similarly, limited information was identified for in situ studies in context of concentrations in aquatic biota and toxicological effects on these organisms.

Fig. II-1. Conceptual model of the triad approach with additional lines of evidence. According to Chapman (1990) and Chapman and Hollert (2006). An integrated approach to evaluate the ecotoxicity of water, sediment, soil and suspended particulate matter, as well as state of contamination in local fish, in combination with chemical analysis of the compartments.
Chapter II – Materials & Methods

In order to limit the scope of this review the focus lies on the Yangtze River mainstream and organic pollutants. Some of the reviewed studies include adjacent water bodies that are linked to the Yangtze River, e.g., tributaries like the Jialing River at Chongqing section or freshwater lakes like Dong Lake in Wuhan. Information about these water bodies are given for comparability reasons. It should also be noted that values have been rounded to whole numbers for reasons of better illustration and comparability. Values below 0.5 have not been set to zero to avoid misinterpretations. Guideline values and some exceptions have not been rounded. All comparisons between the concentrations of pollutants at different sections are based on the reported results, and differences between the laboratories as well as variations in the analysis methods were neglected. Moreover, to assess the worst pollution scenario at each section, not only the average, but also the maximum values were used.

Fig. II-2. Number of publications about the Yangtze River per year (1990-2012). Source: Web of Science, searching for Yangtze River/Changjiang, in article’s topic.
-Part 2-

Ecotoxicological impacts on the Three Gorges Reservoir

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This chapter has been published as parts of two articles in peer-reviewed journals:


Chapter II – Materials & Methods

2 Ecotoxicological impacts on the Three Gorges Reservoir

2.1 Concept

Sediment samples and fish were collected at several sites in the TGR in the administrative region of Chongqing Municipality in three different campaigns as shown in Figure II-3.

In the first phase (September 2011), surface sediment samples were taken at several sites of the TGR mainstream at major tributaries, as well as an artificial lake and a nature reserve in the TGR area, assuming that differing contamination scenarios would be met (Fig. II-4). They were screened for organic contamination by chemical analysis, and effects by acute and mechanism-specific bioassays, in order to identify potential hot-spots and relevant endpoints. Sediment extracts were (a) analyzed for 54 organic compounds selected on basis of the European Water Framework Directive (EWFD Directive 2000/60/EC, EWFD Directive 2008/105/EC), (b) tested in vitro with the Ames fluctuation assay for mutagenicity, (c) the EROD induction assay with RTL-W1 cells for AhR-mediated dioxin-like activity and (d) in vivo with the FET. Further, (e) the bioavailable fraction of particle bound pollutants was tested with native (freeze-dried) sediments in vivo with the SCA. Both in vivo assays were performed with eggs of Danio rerio to screen for embryotoxic and teratogenic impacts. Further, a pre-screening was performed to find a suitable monitoring fish species for the TGR – according to abundance, relevance and feasibility.

In the second phase (May 2012), fish samples of the monitoring species were taken at the same sites at the next possible time point, after the fishing ban in May, and investigated for relevant biomarkers. The in situ investigations of the darkbarbel catfish (Pelteobagrus vachellii, Richardson 1846) comprised (f) genotoxic impacts via micronuclei formation in erythrocytes, as well as hepatic (g) EROD and (h) GST activities. Further, (i) bile of the fish was tested to identify a potential exposure to PAHs, and (j) histopathological alterations in liver and kidney were recorded in parallel. As additional lines of evidence AhR-mediated EROD induction were investigated in vitro and in situ in order to verify the ecological relevance of AhR-mediated modes of action.

In the third phase (May 2013), sediment and fish were sampled in parallel at two hot-spots of particular interest. Sediment was subjected to chemical analysis of 16 PAHs and selected in vitro/in vivo bioassays to determine mutagenicity, EROD induction and
embryotoxicity/teratogenicity. Fish were examined via selected biomarkers for biliary PAH metabolites, genotoxicity, as well as hepatic EROD and GST induction.

Fig. II-3. Scheme of the overall concept displaying the three sampling campaigns (I, II, III) with the respective sampled compartment (sediment/fish), number of sampling sites and individual objectives. Identification and selection of in vitro, in vivo and in situ endpoints was evaluated in course of the ecotoxicological effect assessment; The identification of key pollutants is described in Chapter III-2.2; During the first campaign (I) the darkbarbel catfish Pelteobagrus vachellii was preselected among other species for in situ investigations in the following campaign (II), inter alia due to its demersal lifestyle close to the sediment and frequent occurrence in catches at the TGR (cf. Chapter II-2.2.2).
Fig. II-4. Overview map of sampling locations in the Three Gorges Reservoir area. The Three Gorges Reservoir covers the Yangtze River section between Jiangjin district (Chongqing) and the Three Gorges Dam. Names in grey depict geographical markers: water bodies (italicized), cities and the dam. Fish were sampled in the TGR close to the cities Chongqing (CNG), Fengdu (FEN), Yunyang (YUN) and Wushan (WU), as well as in the TGR watershed in the Hanfeng Lake (HF) and Baijiaxi River (BJX). Labels in black signify sediment sampling locations from campaign September 2011 (with asterisks; CNG-U,-T,-D; FEN-U,-T,-D; YUN-U,-T,-D; WU-U,-T,-D; HF-L; BJX-R) and campaign May 2013 (without asterisks; YAN-A,-B,-C; JIA-A,-B,-C; TGR-A,-B; HAN-A,-B,-C,-D); U = upstream, T = tributary, D = downstream, L = lake, R = reference; A,B,C,D = order in flow direction.
![Diagram showing sampling procedure](image)

**Fig. II-5. Scheme of the sampling procedure for the sites along the TGR mainstream.** At each site along the mainstream three locations were sampled - upstream (U) and downstream (D) the tributary’s inlet, as well as in the tributaries (T). The samples from each location were pooled samples. Each pooled sample consisted of sediment from at least three individual sampling spots at this location, which were combined to reach a total volume of 500 ml. The individual samples were taken in a radius of 50 m from the first sampling spot to cover intrinsic heterogeneity of each sampling location. Arrows depict flow direction of the water; grey filled circles indicate individual sampling spots; white dashed circles display 50 m radius from first sampling spot in the middle comprising all individual spots for one pooled sample.

## 2.2 Sampling

### 2.2.1 Sediment - Campaign September 2011

In a first sampling campaign in September 2011, at a water level of 160 to 166 m altitude above sea level (a.s.l.), the surface layers (~5 cm) of sediments were collected at four major tributaries using a Van-Veen sampler – Jialing River at Chongqing (CNG), Long River at Fengdu (FEN), Pengxi River/Xiao River at Yunyang (YUN), Daning River at Wushan (WU) - along the Yangtze River mainstream of the TGR, as well as the artificial Hanfeng Lake at Kaixian (HF-L) and the Baijiaxi River in the Pengxi River Wetland Nature Reserve (BJX-R) in the TGR watershed (**Fig. II-4**). The Pengxi River Wetland Nature Reserve was selected as reference site, assuming minimum pollution in the poorly industrialized area. At each site along the
Chapter II – Materials & Methods

mainstream three locations were sampled - upstream (U) and downstream (D) the tributary’s inlet, as well as in the tributaries (T) (Fig. II-5). At the artificial lake site and the reference site only one location was sampled. The samples from each location were pooled samples. Each pooled sample consisted of sediment from at least three individual sampling spots at this location, which were combined to reach a total volume of 500 ml. The individual samples were taken in a radius of 50 m from the first sampling spot to cover intrinsic heterogeneity of each sampling location. The pooled samples were stored in inert PTFE-bottles at 4°C for transportation. The samples were freeze dried, sieved with a 2 mm sieve to remove sticks and stones, and kept in amber glass bottles at 4°C for further analysis according to the methods given by Hollert et al. (2000).

2.2.2 Fish - Campaign May 2012

In a second sampling campaign in May 2012, at a water level of 164-160 m altitude a.s.l., fish samples were taken at the sampling sites Chongqing (CNG 2012), Fengdu (FEN 2012), Yunyang (YUN 2012) and Wushan (WU 2012) in the TGR, as well as the Hanfeng Lake (HF 2012) and Baijiaxi River (BJX 2012) in the TGR watershed (Fig. II-4). The Baijiaxi River in the Pengxi River Wetland Nature Reserve was again selected as reference site. The darkbarbel catfish (*Pelleobagrus vachellii*, Richardson 1846), was chosen as monitoring fish species due to its demersal lifestyle and low to medium migration – thus being suitable to indicate local contamination over longer periods of time -, as well as its distribution all along the TGR and other sections of the Yangtze River. Furthermore, it frequently occurred in catches from all sampled sites and has a major economic importance (Ministry of Environmental Protection - China 2007). At each site 10 fish of comparable size (mean ± SD: total length 165 ± 15 mm; standard length 142 ± 14 mm; weight of 40 ± 11 g; indifferent sex) (Table II-1) were sampled with help from local fishermen. The fish were kept in water of the local water body in an aerated tank till each fish was euthanized with clove oil, followed by an additional blow to the head. Blood samples were obtained from a caudal section with a heparinized syringe and transferred to two microscope slides per fish according to the methods given by Rocha et al. (2009) and Boettcher et al. (2010). Each blood smear was fixed in methanol for 5 min. Bile samples were obtained from the gall bladder with a syringe, transferred to 2 mL cryovials and stored at 4°C. Excised liver samples were divided, with one half immediately transferred to a 2 mL cryovial and stored in liquid nitrogen at -80°C. The other half was fixed in 10 % buffered formalin and stored at room temperature.
Table II-1. Main morphological parameters of *Pelteobagrus vachellii* from sampling campaign May 2012 and 2013. The Fulton condition factor (k-value) was calculated according to Barnham and Baxter (1998) based on Fulton (1902) and Ricker (1975) with total length (tot) and standard length (std) (cf. Chapter II-2.7.1, Equation II-3); Values are stated as means with standard deviation; 2012 samples n=10; 2013 samples n=20.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Total length [mm]</th>
<th>Standard length [mm]</th>
<th>Weight [g]</th>
<th>k-value (tot)</th>
<th>k-value (std)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNG 2012</td>
<td>154 ± 17</td>
<td>132 ± 15</td>
<td>37 ± 12</td>
<td>1.01 ± 0.08</td>
<td>1.59 ± 0.13</td>
</tr>
<tr>
<td>FEN 2012</td>
<td>172 ± 12</td>
<td>148 ± 12</td>
<td>39 ± 9</td>
<td>0.75 ± 0.08</td>
<td>1.20 ± 0.11</td>
</tr>
<tr>
<td>YUN 2012</td>
<td>174 ± 7</td>
<td>149 ± 6</td>
<td>42 ± 6</td>
<td>0.81 ± 0.05</td>
<td>1.27 ± 0.09</td>
</tr>
<tr>
<td>WU 2012</td>
<td>156 ± 11</td>
<td>134 ± 8</td>
<td>34 ± 7</td>
<td>0.90 ± 0.13</td>
<td>1.41 ± 0.17</td>
</tr>
<tr>
<td>HF 2012</td>
<td>167 ± 13</td>
<td>145 ± 11</td>
<td>45 ± 12</td>
<td>0.95 ± 0.09</td>
<td>1.46 ± 0.14</td>
</tr>
<tr>
<td>BJX 2012</td>
<td>166 ± 19</td>
<td>144 ± 20</td>
<td>45 ± 15</td>
<td>0.96 ± 0.12</td>
<td>1.48 ± 0.15</td>
</tr>
<tr>
<td>CNG 2013</td>
<td>177 ± 15</td>
<td>152 ± 13</td>
<td>54 ± 15</td>
<td>0.96 ± 0.12</td>
<td>1.54 ± 0.23</td>
</tr>
<tr>
<td>HF 2013</td>
<td>201 ± 24</td>
<td>175 ± 21</td>
<td>69 ± 24</td>
<td>0.83 ± 0.10</td>
<td>1.27 ± 0.18</td>
</tr>
<tr>
<td>Mean 2012</td>
<td>165 ± 15</td>
<td>142 ± 14</td>
<td>40 ± 11</td>
<td>0.90 ± 0.13</td>
<td>1.40 ± 0.18</td>
</tr>
<tr>
<td>Mean 2013</td>
<td>189 ± 23</td>
<td>163 ± 21</td>
<td>61 ± 21</td>
<td>0.90 ± 0.13</td>
<td>1.40 ± 0.25</td>
</tr>
</tbody>
</table>

2.2.3 Sediment & fish - Campaign May 2013

In a third sampling campaign in May 2013, at a water level of 160 m altitude a.s.l., sediment and fish samples were taken in parallel at two identified regional hot-spots – near the cities of Chongqing (CNG 2013) and Kaixian (HF 2013) (cf. Chapter III-2.6). To ensure the same biological status of the fish according to their seasonal rhythm, in order to prevent major physiological oscillations that could influence the biomarkers, and because May and September have shown comparable temperature, precipitation and TGR water level, May was chosen for the combined sampling of sediment and fish. For sediment, at Chongqing three samples were taken in the Jialing River (JIA), three samples in the Yangtze River (YAN) upstream of the conversion zone of both rivers, and two samples downstream of the conversion zone in the reservoir (TGR). Samples were labelled in order of flow direction (A, B, C) (Fig. II-4). In addition four samples were taken in the Hanfeng artificial lake (HF) at Kaixian. Samples were labelled in order of flow direction (A, B, C, D) (Fig. II-4). For fish, 20 individuals of comparable size (mean ± SD: total length 189 ± 23 mm; standard length 163 ± 21 mm; weight 61 ± 21 g; indifferent sex) (Table II-1) were sampled at both sites (CNG; HF) (Fig. II-4). Sampling procedures for sediment and fish, as well as further treatment were as described before.
2.3 Chemicals and material

Acetone (≥99.5%, p.a.), n-hexane (≥99%, p.a.), dichloromethane (≥99.8%, p.a.), isoctane (≥99%, p.a.), cyclohexane (≥99.5%, p.a.), hydrochloric acid (37%, ACS reagent), ethanol (≥99.8%), 3,4-dichloroaniline (DCA), 7-ethoxresorufin, fluorescamine, reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), reduced L-glutathione, 1-chloro-2,4-dinitrobenzene (CDNB) and acridine were purchased from Sigma Aldrich GmbH (Deisenhofen, Germany) and methanol (99%) from Sigma Aldrich Inc. (Shanghai, China). 2,3,7,8-tetrachlorodibenzo-p-dioxin was purchased from LGC Promochem GmbH (Wesel, Germany). Dimethylsulfoxide (DMSO), aluminium oxide (for column chromatography) and sodium sulfate (≥99%, p.a.) were supplied by Carl Roth GmbH & Co. KG (Karlsruhe, Germany), silica gel 60 (size: 0.063-0.200 mm) and acetonitrile (gradient grade, p.a.) by Merck group (Darmstadt, Germany), S9 fraction (from phenobarbital/β-naphthoflavon treated rats) by Harlan Cytotest Research GmbH (Rossdorf, Germany), heparine from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), cleaned silica sand by Büchi Labortechnik AG (Flawil, Switzerland) and Quartz sand (grain size W4) by Quarzwerke GmbH (Frechen, Germany). Amber glass bottles and vials, PTFE bottles, syringes, cryovials and microscope slides were purchased from VWR International GmbH (Darmstadt, Germany), 24-, 96- and 384-well microplates from TPP Techno Plastic Products AG (Trasadingen, Switzerland) and gas permeable foil from Renner GmbH (Dannstadt, Germany).

2.4 Sediment extraction

Each freeze dried sediment sample was extracted with acetone-hexane (1:1) (p.a.) in a pressurized liquid extractor (Speed Extractor E-916, Büchi Labortechnik AG, Flawil, Switzerland) at 100°C and 120 bar in two cycles (heat up 1 min; hold 10 min; discharge 2 min; flush with solvent 1 min; flush with gas 4 min). For process controls, extraction tubes were filled with cleaned silica sand and further processed in parallel. After the reduction in volume with a rotary evaporator and under a gentle nitrogen stream, the extracts were redissolved in dimethylsulfoxide (DMSO) to a final concentration of 20 g sediment equivalents (SEQ) per mL DMSO and stored at 4°C in amber glass vials for further testing. The whole process was performed under protection from light to prevent photodegradation of toxic compounds. In the following, sediment concentrations in gram SEQ per milliliter solvent/medium will be given as gram per milliliter (g/mL).
2.5 Triad A: Chemistry

2.5.1 Chemical analysis

_Sediment - Campaign September 2011_

Every extract was screened for 54 organic priority compounds (Table II-2), selected on basis of the European Water Framework Directive (EWFD Directive 2000/60/EC, EWFD Directive 2008/105/EC), according to Erger et al. (2012). The analysis was performed at the IWW Rhenish-Westfalian Institute for Water Research, Mülheim a.d. Ruhr, Germany. Per sample 20 g freeze dried sediment were extracted as described before, with process controls in parallel. After reduction in volume the extracts were cleaned up chromatographically on columns filled with 2 g silica gel and 2 g aluminium oxide using 15 mL n-hexane, 5 mL n-hexane: dichloromethane (9:1) and 20 mL n-hexane: dichloromethane (4:1) as eluate. After adjustment to 200 μl each sample was provided for gas chromatography–mass spectrometry (GC-MS) analysis using an GC-MS system (6890 GC and 5973 mass selective detector (MSD) single quadrupole mass analyzer) equipped with an automatic sampler (MPS 2) (Agilent Technologies GmbH, Böblingen, Germany) and Cooled Injection System (CIS 4, Gerstel GmbH, Mülheim a.d. Ruhr, Germany). The limit of detection (LOD) was 10 ng PAH/g SEQ. In the following, PAH concentrations in nanogram PAH per gram SEQ will be given as nanogram per gram (ng/g). Given are mean values, with standard deviation (± SD) if applicable.

<table>
<thead>
<tr>
<th>Table II-2. Target compounds in sediment extracts of campaign September 2011 (a) and May 2013 (b).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OCPs</strong></td>
</tr>
<tr>
<td><strong>PAHs</strong></td>
</tr>
<tr>
<td><strong>PBDEs</strong></td>
</tr>
<tr>
<td><strong>PCBs</strong></td>
</tr>
<tr>
<td><strong>Other intermediate and by-products</strong></td>
</tr>
</tbody>
</table>

*Priority PAHs according to the Environmental Protection Agency & Office of the Federal Registration - USA (1982)*
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Sediment - Campaign May 2013

Based on the results of the prior 2011 campaign sediment extracts of campaign May 2013 were screened only for the 16 priority PAHs (Table II-2), according to International Standard Operation (ISO) guideline 18287:2006. Per sample 20 g freeze dried sediment were extracted as described before, with process controls in parallel. Activated copper was used to remove sulphur over 96h. After reduction in volume the samples were cleaned up chromatographically on columns filled with 4 g silica gel and 3 g sodium sulfate using n-hexane (p.a.) as eluate – instead of petroleum ether. Isooctane (p.a.) was added as retention solvent, before the extract was reduced in volume under a gentle nitrogen stream. The extract was set to a final concentration of 20 g/mL with cyclohexane (p.a.) before been provided for GC-MS analysis. The GC system (GC 7890 A) was coupled with a MSD (5975 C inert XL MSD with Triple-Axis-Detector) operated in SIM (Selective Ion Monitoring) mode and an autosampler (Agilent Technologies GmbH, Böblingen, Germany). The LOD was 10 ng PAH/g SEQ. In the following, PAH concentrations in nanogram PAH per gram SEQ will be given as nanogram per gram (ng/g). Given are mean values, with standard deviation (± SD) if applicable.

2.5.2 Total organic carbon analysis

Per sample 150 to 300 mg freeze dried sediment were transferred to amber glass vials and 2 mL hydrochloric acid (2 M) per gram sediment were added. After evaporation in the fume hood for 24 h, samples were dried at 105°C overnight. Per sample 3 individual replicates of 25 mg each were analyzed for total organic carbon (TOC) content in a Vario EL III Elemental Analyzer and results were computed with Winvar V5.01 (Elementar Analysensysteme GmbH, Hanau, Germany). Given are mean values, with standard deviation (± SD) if applicable.

2.5.3 Principal Component Analysis

Principal component analysis (PCA) as an ordination technique can be deployed in a broad set of data to reduce the number of dimensions of the multivariate dataset (m-sites x n-compounds) to its ‘principal components’. The total complexity is condensed so that a few axes explain most of the variance of the dataset in order to generate hypotheses on the relationship between the sites characteristics and the content of the individual substances (Cincinelli et al. 2007, Ottermanns 2008, Höss et al. 2010).

The PCA was chosen after consulting the results of Detrended Correspondence Analysis (DCA), which indicated very short gradients (1.067 and 0.673 for the first and second axis
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respectively for the 2011 data, 0.900 and 0.703 for the 2013 data) and thus allows for the use of a linear method (Ottermanns 2008). PCA analysis was conducted by means of Canoco for Windows, Version 4.5 (Biometris, Plant Research International, Wageningen, 2002). The data was log-transformed prior to the analysis, which was performed on the covariance matrix, because the variance of the pollutant concentrations was considered sufficiently homogenous.

2.5.4 PAH source analysis

Well suited pairs of parent PAHs - with the same molecular mass (M) - for diagnostic ratios are anthracene (ANT) and phenanthrene (PHE) (M: 178 g/mol), fluoranthene (FLA) and pyrene (PYR) (M: 202 g/mol), benzo[a]anthracene (BaA) and chrysene (CHR) (M: 228 g/mol), as well as indeno[1,2,3-cd]pyrene (IcdP) and benzo[g,h,i]perylene (BghiP) (M: 276 g/mol) (Yunker et al. 2002). Diagnostic ratios are given in Table II-3. The ANT/ANT+PHE ratio is recommended to distinguish petroleum sources (petrogenic) from combustion sources (pyrogenic), while the FLA/FLA+PYR ratio separates petroleum combustion from other combustion types. Further, the BaA/BaA+CHR and the IcdP/IcdP+BghiP ratios are applicable to support a refined identification of the combustion sources (Yunker et al. 2002, Tobiszewski & Namieśnik 2012). For the evaluation of the sediment samples all four ratios were determined in order to corroborate the individual results.

Table II-3. Diagnostic ratios for potential PAH sources.

<table>
<thead>
<tr>
<th>PAH ratio</th>
<th>Values</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANT/(ANT+PHE)</td>
<td>&lt;0.1</td>
<td>petroleum combustion</td>
<td>(Pies et al. 2008)</td>
</tr>
<tr>
<td></td>
<td>&gt;0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLA/(FLA+PYR)</td>
<td>&lt;0.4</td>
<td>petroleum vehicular emission</td>
<td>(De la Torre-Roche et al. 2009)</td>
</tr>
<tr>
<td></td>
<td>0.4-0.5</td>
<td>coal/grass/wood combustion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BaA/(BaA+CHR)</td>
<td>&lt;0.2</td>
<td>petroleum combustion</td>
<td>(Yunker et al. 2002)</td>
</tr>
<tr>
<td></td>
<td>0.2-0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IcdP/(IcdP+BghiP)</td>
<td>&lt;0.2</td>
<td>liquid fossil fuel combustion</td>
<td>(Yunker et al. 2002)</td>
</tr>
<tr>
<td></td>
<td>0.2-0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;0.5</td>
<td>coal, grass, wood combustion</td>
<td></td>
</tr>
</tbody>
</table>
2.6 Triad B: *In vitro/in vivo* bioassays

2.6.1 Ames fluctuation assay with *Salmonella typhimurium*

The Ames fluctuation assay is a modified version of the traditional Ames test (Ames et al. 1975, McCann et al. 1975). While the traditional test is performed using agar plates (plate incorporation technique), the Ames fluctuation assay uses a liquid medium to expose and incubate bacteria in 24- and 384-well microplates, respectively. The Ames fluctuation assay including cytotoxicity determination was performed according to the method described by the ISO guideline 11350:2012 as detailed by Reifferscheid et al. (2012) and Higley et al. (2012). Each sample was tested in three individual replicates.

Two tester strains were used to examine the samples’ mutagenic potential. Both strains are auxotrophic mutants of the bacterium *Salmonella typhimurium*. While TA98 carries a frameshift mutation (hisD3052), TA100 carries a base pair substitution (hisG46). Both mutations disable the bacteria from growing in histidine-free medium unless they are reverted to a prototrophic state by back mutation (Ames et al. 1975). To determine metabolic activation of promutagenic substances each sample was tested additionally with a liver homogenate S9-fraction from phenobarbital/β-naphthoflavon treated rats, further supplemented for application in the assay (S9 supplement). Based on the results of the prior 2011 campaign sediment samples of campaign May 2013 were only tested with strain TA98 in the presence and absence of S9 supplement for metabolic activation.

Bacteria were grown overnight in Oxoid broth no.2 supplemented with ampicillin (50 mg/L) at 37 ± 1°C shaking at 150 rpm. After 9:45 h of incubation, optical density ($\lambda=595$ nm) was measured for both bacteria cultures. They were then diluted with exposure medium, containing L-histidine (1 mg/L), to 180 (TA98) and 45 (TA100) formazine attenuation units (FAU). Six concentration levels per sample were prepared by 1:2 dilution steps. While in all tests DMSO was used as negative control with a maximum of 2% per well, different positive controls where used depending on the used tester strain and the presence or absence of S9 supplement. 4-nitro-o-phenylenediamine (6.53 x 10⁻⁵ M) was used for testing TA98 without S9 supplement. Nitrofurantoin (1.05 x 10⁻⁶ M) was used for testing TA100 without S9 supplement. 2-aminoanthracene was used for testing TA98 (5.17 x 10⁻⁷ M) and TA100 (2.06 x 10⁻⁶ M) with S9-activity. In 24-well microplates 10 μL of each concentration level, negative control or positive control were added to 490 μL of bacteria suspension. All samples were preexposed for 100 min at 37 ± 1°C and shaking at 150 rpm. After the preincubation, 2.5 mL of the histidine-
deficient reversion indicator medium, containing the pH indicator dye bromocresol purple, were added to each well. Afterwards, 50 μL each were transferred into 48 wells per replicate (sample dilutions and controls) on 384-well microplates which were incubated for additional 48 h at 37 ± 1°C without agitation.

Only reverted prototrophic bacteria were able to grow in the histidine-free reversion indicator medium while their metabolic activity caused acidification of the medium. Acidification in the wells was detected optically by a color shift of the reversion indicator medium from purple to yellow. All wells that indicated reversions were counted for each sample concentration, negative control and positive control.

Tests were valid when mean values of revertants were > 0 and ≤ 10 per 48 wells in negative controls and ≥ 25 per 48 wells in positive controls. No Observed Effect Concentrations (NOEC) were statistically determined as described in Chapter II-2.8. In the following, test concentrations in milligram SEQ per milliliter solvent will be given as milligram per milliliter (mg/mL).

2.6.2 EROD induction assay with RTL-W1 cells

To determine the dioxin-like activity of the sediment extracts the EROD induction assay was conducted according to Wölz et al. (2008), based on the procedure described by Behrens et al. (1998). In order to avoid cytotoxic effects in the EROD assay, the neutral red retention assay (NR) was performed beforehand to assess the initial concentrations of the sediment extracts set by the NR80 value (80% viability). The NR assay was based on the method described by Babich and Borenfreund (1992). Both assays were performed with the CYP1A expressing fibroblast-like permanent cell line RTL-W1 from primary hepatocytes of rainbow trout (Oncorhynchus mykiss) (Lee et al. 1993). The cells - kindly provided by Drs. Niels Bols and Lucy Lee from the University of Waterloo, Canada - were cultured according to the procedure described by Klee et al. (2004). Samples were tested in three independent replicates, each with three internal replicates serially diluted with medium in seven 1:2 steps. As positive control 2,3,7,8-tetrachlorodibenzo-\( p \)-dioxin was used in a test concentration range from 3.13 to 100 pM. To avoid cytotoxicity, the maximum DMSO concentration per well was restricted to 1%.

In short, cells were seeded in 96-well microtiter plates and allowed to grow to 100% confluence for 72 h at 20°C. After removal of the medium, cells were exposed to the diluted sediment extracts for 72 h at 20°C to induce the EROD production. After termination of the EROD induction, by removal of the growth medium and freezing the cells at -80°C to lyse the cells
and release the enzymes, exogenous 7-ethoxyresorufin substrate was added under protection from light. To allow enzyme and substrate to form energetically favorable complexes they were preincubated for 10 min at room temperature in the dark. Subsequently, the enzymatic deethylation of the substrate to resorufin was started by adding NADPH. After a reaction period of 10 min under the same incubation conditions it was stopped by the addition of fluorescamine dissolved in acetonitrile. After further 15 min, the amount of produced resorufin was determined fluorometrically in a spectrophotometer (Infinite M200, Tecan, Crailsheim, Germany) at excitation 544 nm and emission 590 nm. The protein amount was measured fluorometrically applying the fluorescamine method by Kennedy and Jones (1994) at excitation 360 nm and emission 465 nm. The enzyme activity was computed based on the quantity of produced resorufin per total amount protein and reaction time. The concluding concentration response curves for EROD induction were calculated by nonlinear regression (Prism 5.0, GraphPad Software Inc., San Diego, USA) applying the classic sigmoid curve or Boltzmann curve as model equations.

To provide comparability, EC25 values of the EROD induction of each sample replicate (EC25 sample) were related to the EC25 of the maximum response in the corresponding TCDD standard curve (EC25 TCDD) to compute the bioassay-derived toxic equivalents (bio-TEqs) (Equation II-1), according to the fixed effect level quantification method (Engwall et al. 1996).

\[
\text{bio-TEQ} \left[ \frac{\text{pg TCDD}}{g \text{ SEQ}} \right] = \frac{\text{EC25 TCDD} \left[ \frac{\text{pg TCDD}}{mL} \right]}{\text{EC25 sample} \left[ \frac{\text{g SEQ}}{mL} \right]}
\]

(Equation II-1)

The EC25 was used for means of calculation, because the EC50 was not well defined by the dose-response curves of several samples. Hence, the EC25 was considered more reliable, as suggested by Engwall et al. (1996). In the following, bio-TEQ values in picogram TCDD per gram SEQ will be given as picogram per gram (pg/g). Given are mean values, with standard deviation (± SD) if applicable.

2.6.3 Calculation of chem-TEQ values

As described in Chapter III-2.2.1, based on chemical analysis only 16 priority PAHs - out of 54 analyzed organic compounds - could be detected in the sediments of sampling campaign September 2011. Their ubiquitous presence could be verified in sediment of campaign May
2013. Since the AhR is highly inducible by coplanar ligands (Safe 1990, Tanguay et al. 1999, Karchner et al. 2005), the also coplanar PAHs were considered to be potential inducers of the recorded EROD induction. To evaluate their contribution to the overall Ah receptor mediated activity given as bio-TEQs, toxic equivalents derived from chemical analysis (chem-TEQs) were calculated (Equation II-2). Therefore, concentrations of the PAHs were referred to their specific relative potencies (REP) in the EROD induction assay with RTL-W1 cells according to Bols et al. (1999). REPs are the cell line specific potencies to trigger an effect relative to a strong inducer, here TCDD. In the following, chem-TEQ values in picogram total PAH (PAH$_{216}$) per gram SEQ will be given as picogram per gram (pg/g).

$$chem-TEQ \left( \frac{pg \ PAH_{216}}{g \ SEQ} \right) = \sum_{i=1}^{n} \frac{conc. \ PAH_i}{g \ SEQ} \times REP_i$$

(Equation II-2)

2.6.4 Maintenance of Danio rerio and egg production

Maintenance of Danio rerio was conducted according to Braunbeck et al. (2005) with modifications previously described by Peddinghaus et al. (2012). The zebrafish were kept in glass aquaria at a water temperature of 26 ± 1°C, a pH value of 7.8, a hardness of about 11°dH and a constant day to night rhythm (14/10h). They were fed with commercially dry flake food (TetraMin™ flakes; Tetra, Melle Germany) and living nauplius larvae of Artemia sp. (Silver Star Artemia, Inter Ryba GmbH, Zeven, Germany) once per day ad libitum. For egg production groups of twenty 3-month old zebrafish with a ratio of 3:2 (males: females) were kept per aquarium. Spawning trays were placed into the aquaria for egg collection. They consisted of a flat glass basin and a metal net cover with attached artificial plants, which serve as a breeding stimulant and substrate. The metal net prevented the fish from predating on their own spawn. Within 30 min after the onset of light in the morning mating, spawning and fertilization took place (Westerfield 2007).

2.6.5 Fish Embryo Toxicity Test with Danio rerio

The Fish Embryo Toxicity Test (FET) was performed according to the German Standard guideline DIN 38415-6 (2009) and the corresponding draft OECD guideline (OECD draft 2006) with Danio rerio over a prolonged testing period of 96 h. The aim was to determine the embryotoxic and teratogenic potential of the sediment extracts.
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The FET was started no later than 3 h past fertilization (128-cell stage). Fertilized freshly spawned eggs were identified with a stereo microscope (SMZ 1500; Nikon GmbH, Düsseldorf, Germany), at a minimum magnification of 25x, on basis of the following criteria: The chorion surrounded the perivitelline space, which contained the yolk, and the blastodisc was located at the animal pole of the yolk. Exclusively normally developed eggs at minimum 8-cell stage were chosen for application in the FET. Till further testing eggs were transferred into artificial water (ISO 7346-3:1996) at 26 ± 1°C to guarantee homogenous water quality and ion concentration in each test series.

Sediment extracts were diluted with DMSO in five steps and added to the artificial water. Thereby, the highest tested concentration for all samples was 100 mg SEQ/mL and all dilution steps were adjusted to a DMSO concentration of 0.5%. Preliminary experiments with DMSO and artificial water displayed no differences in the results. Thus, artificial water in the absence of the extracts was applied as negative control (validity criteria: mortality <10%). As solvent control, DMSO was tested in a concentration of 0.5% in artificial water. Further, 3.7 mg/L DCA dissolved in ultrapure water (ZFMQ 23004, Millipore, Molsheim, France) in absence of the extracts was used as standard positive control (validity criteria: mortality >10%). Subsequently, eggs were transferred to 24-well plates with 2 mL test solution and controls per well. To prevent interactions between adjacent wells, each plate was covered with a gas permeable foil and incubated at 26 ± 1°C for 96h using the static test design.

Lethal and sublethal effects were evaluated with an inverted microscope (Eclipse TS 100, Nikon GmbH, Düsseldorf, Germany) at magnifications of 40x and 100x. The following criteria were assessed as lethal endpoints according to DIN EN ISO 38415-6 (2009): (a) coagulation of the embryo, (b) no heartbeat (c) non-detachment of the tail. In addition to that, sublethal endpoints were: (d) weak heartbeat, (e) weak or (f) no blood circulation, (g) edema, an (h) underdeveloped or (i) malformed embryo, (j) missing eye primordia, (k) weak or (l) no pigmentation of the body, (m) missing eye pigmentation, (n) curved or (o) deformed spine and (p) malformed or (q) missing fins. Selected developmental stages and sublethal effects of Danio rerio are shown in Figure II-6. Each test series was scored valid when the negative control did not exceed 10% mortality and the positive control showed more than 10% mortality.

Each extract from sampling campaign September 2011 was tested in three independent replicates with ten eggs per test concentration. Extracts from campaign May 2013 were not investigated in detail in the FET. Based on a pre-screening of the highest concentration (100 mg SEQ/mL) and with regard to results from other bioassays on the same samples, it was
decided that no significant effects could be expected. Thus, to avoid unnecessary usage of fish embryos the samples were not investigated any further.

Results were analyzed according to lethality and overall effects (lethal plus sublethal). The concluding concentration response curves were plotted by nonlinear regression (Prism 5.0, GraphPad Software Inc., San Diego, USA) applying the classic sigmoid curve or Boltzmann curve as model equations to calculate LC50 and EC50 values with 95% confidence intervals (CI). In the following, LC50 and EC50 values in milligram SEQ per milliliter water will be given as milligram per milliliter (mg/mL).

2.6.6 Sediment Contact Assay with Danio rerio

The Sediment Contact Assay (SCA) was performed on basis of the German standard guideline DIN 38415-6 (2009), adapted for sediment testing as described by Hollert et al. (2003) and detailed by Zielke et al. (2011). The aim was to examine the embryotoxic and teratogenic potential of bioavailable fractions of particle-bound pollutants in native (freeze-dried) sediment (cf. Kosmehl et al. 2007). Initially, samples were prescreened at the highest sediment concentration (100%) to identify possible inducing samples.

3 g sediment per sample was transferred into a 20 mL crystallization glass vessel in the afternoon prior to testing and according to the initial wet weight artificial water was added to the freeze-dried sediment to reconstitute the original state. In addition, 4 mL artificial water was supplemented to each batch. Negative controls consisted firstly of solely 4 mL artificial water (water control) and secondly 3 g quartz sand with 4 mL artificial water (sand control). Analogous to this, positive controls consisted of 4 mL DCA (3.7 mg/L) in artificial water (water control) and quartz sand with DCA (3.7 mg/L) (sand control). Each batch was covered with a gas permeable foil and preincubated at 26 ± 1°C overnight on a horizontal shaker at 50 rpm to allow oxygen saturation in the water column. On the following day selection of the eggs was performed according to the FET procedure. Per vessel five normally developed eggs - between 8-cell and 128-cell stage – were added in 1 mL artificial water to the batch and incubated at 26 ± 1°C and 50 rpm for 96 h.

Each sample was tested in three independent replicates, each with three parallel replicates, with five fish eggs per parallel replicate. Controls were tested in four parallel replicates with each five fish eggs. Lethal and sublethal criteria were evaluated according to the FET.
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Freeze-dried sediment from campaign May 2013 was not investigated in detail in the SCA as it was decided that - based on prescreening of the corresponding sediment extracts in the FET and with regard to results from other bioassays on the same samples - no significant effects could be expected. Thus, to avoid unnecessary usage of fish embryos the samples were not investigated any further.

Fig. II-6. Selected developmental stages (A, B, C) and sublethal effects (D,E,F) of Danio rerio. Normally developed embryo after 24 h (A), 48 h (B) and 72 h before hatching (C); embryo with edema (arrow) after 48 h (D); hatched larvae with curved spine (arrow) after 72 h (E); hatched larvae with curved spine (upper arrow) and edema (lower arrow) after 96 h (F).
2.7 Triad C: In situ biomarkers

2.7.1 Fulton condition factor

The Fulton condition factor (k-value) can be consulted to judge the long-term condition of fish. It was calculated according to Barnham and Baxter (1998) based on Fulton (1902) and Ricker (1975) (Equation II-3). The weight \( W \) was given in gram and the standard/total length \( L \) was given in mm. Given are mean values, with standard deviation (± SD) if applicable.

\[
k = \frac{10^5 W}{L^3}
\]

(Equation II-3)

2.7.2 Micronucleus assay with erythrocytes

The micronucleus assay was performed with minor modifications according to the methods given in Rocha et al. (2009) and Boettcher et al. (2010). To determine micronucleus formation rate acridine orange was used for staining of the fixed blood smears on the slides. Per fish 4000 erythrocytes (2000 per slide) were examined using an epifluorescence microscope (BIOREVO BZ-9000E, Keyence, Neu-Isenburg, Germany) equipped with an oil-immersion lens at a 600x magnification. The cells were evaluated according to the scoring criteria of the ISO guideline 21427-2:2006: (a) only erythrocytes with intact cell membrane were scored. The micronuclei should have (b) a maximum size of about 30% of and (c) the same staining intensity as the main nucleus, as well as (d) a clear separation from it. A representative erythrocyte with micronucleus is shown in Fig. II-7. Further, induction factors (IF) were computed, which give the induction of the mean micronucleus frequency of each sample referred to the reference site.

Fig. II-7. Micrograph of an erythrocyte with micronucleus (arrow). Nuclei and micronuclei were stained using acridine orange dye.
2.7.3 Hepatic biotransformation enzymes

Determination of hepatic EROD activity

Homogenization and centrifugation of excised liver samples was performed according to Bonacci et al. (2003) to prepare enzymatic active liver fraction (S9) as described by Kammann et al. (2014). All samples were kept on ice during the complete procedure. Small pieces of deep frozen liver samples were added to cold dipotassium phosphate buffer (pH 7.5) at a ratio of 1:10 (w/v) and homogenized with an electric disperser (VDI 12, VWR, Darmstadt, Germany). The homogenates were centrifuged at 9000×g at 4°C for 20 min (Rotina 420R, Hettich, Tutlingen, Germany) and subsequently the supernatant (S9) was collected.

The activity of 7-ethoxy-resorufin-O-deethylase was measured with the fluorometric method of Maria et al. (2005) as described by Kammann et al. (2014). 1 mL freshly prepared 0.5 µM 7-ethoxresorufin solution at room temperature was mixed with 100 µL cold S9 fraction in a quartz cuvette. The reaction was started by adding 10 µL freshly prepared 10 mM NADPH solution. The fluorescence of the reaction product resorufin was recorded at room temperature for 5 min in 10 seconds intervals at excitation 530 nm and emission 585 nm in a spectrofluorometer (Jasco FP-750, Gross Umstadt, Germany). Each sample was measured in duplicate. To determine the specific EROD activity fluorescences were referred to a resorufin reference curve and the conversion rate of the blank (dipotassium phosphate buffer instead of S9 fraction) was subtracted. All samples were kept on ice during the complete procedure till measurement. The specific enzyme activity was expressed as picomol resorufin produced per milligram total protein and minute (pmol/mg/min). Further, induction factors (IF) were calculated, which give the induction of the mean EROD activity of each sample referred to the reference (BJX 2012). If not stated otherwise, values given are mean values, with standard deviation (± SD) if applicable.

Determination of hepatic GST activity

Homogenization and centrifugation of excised liver samples was performed as described above. The activity of glutathione S-transferase was measured according to Habig et al. (1974) as described by Kammann et al. (2014). 750 µL 0.1 M sodium phosphate buffer (pH 6.5) were mixed with 30 µL 25 mM CDNB in ethanol, 45 µL 50 mM dipotassium phosphate buffer (pH 7.5) at room temperature and 15 µL cold S9 fraction. The reaction was started with 75 µL 11 mM reduced glutathione solution. The absorbance was measured at 25°C for 5 min in 5 seconds intervals at 340 nm in a spectrophotometer (Infinite M200, Tecan, Crailsheim,
Germany). Each sample was measured in duplicate. The change of the substrate was calculated from the change of absorbance applying the Lambert-Beer law after subtraction of the conversion rate of the blank (dipotassium phosphate buffer instead of S9 fraction). The specific enzyme activity was expressed as nanomol conjugated 1-chloro-2,4-dinitrobenzene per milligram total protein and minute (nmol/mg/min). Further, induction factors (IF) were calculated, which give the induction of the mean GST activity of each sample referred to the reference (BJX 2012). If not stated otherwise, values given are mean values, with standard deviation (± SD) if applicable.

**Determination of protein concentrations**

Protein concentrations of the S9 fractions were determined photometrically with a bicinchoninic acid protein assay kit (Sigma Aldrich GmbH, Deisenhofen, Germany). The assay is based on the Lowry procedure. Reaction solution (reagent A to reagent B - 50:1) was freshly prepared and 200 μL were added to 25 μL of different dilutions of S9 fraction and incubated for 30 min at 37°C. Absorbances were recorded at 562 nm in a spectrophotometer (Infinite M200, Tecan, Crailsheim, Germany). To determine protein concentrations absorbances were referred to a bovine serum albumin reference curve and the absorbance rate of the blank (dipotassium phosphate buffer instead of S9 fraction) was subtracted.

**2.7.4 Analysis of PAH metabolites in bile**

Fish bile of *Pelteobagrus vachellii* from campaign May 2012 were analyzed for PAH metabolites 1-hydroxypyrene (1-OHPyr) and 1-hydroxyphenanthrene (1-OHPhe) according to the protocol given by Kammann (2007). Due to low bile volumes available samples had to be combined into 3 pooled samples per site to reach the minimum required volume (25 μL), with exception of WU where bile was only sufficient for 2 pooled samples. Available bile samples were n=7 at WU, n=9 at FEN and the reference site BJX, and n=10 at CNG, YUN and HF. Absorption of bile at 380 nm was applied to normalize the 1-hydroxypyrene level. Fish of campaign 2013 did not supply sufficient bile volumes, thus required minimum amounts for analysis were not achieved.

In the following, PAH metabolite concentrations in nanogram 1-OHPyr or 1-OHPhe per milliliter bile will be given as nanogram per milliliter (ng/mL). The 1-OHPyr/1-OHPhe ratio will be given in dimensionless units. The absorption at 380 nm (dimensionless units) per milliliter bile will be given as absorption per milliliter (A380/mL). If not stated otherwise, values given are mean values, with standard deviation (± SD) if applicable.
2.7.5 Histopathological evaluation

Fixed samples of excised liver and kidney with surrounding tissue of *Peleobagrus vachellii* from sampling campaign May 2012 were investigated with regard to histopathological alterations. Fixed samples were paraffin-embedded and routinely processed for histological examination, sections of 4 μm thickness were cut. Sections were stained with haematoxylin-eosin (H&E) and examined by light microscopy. Histopathological changes of liver and kidney were graded as 0 (no), 1 (scattered), 2 (mild), 3 (mild to moderate), 4 (moderate), 5 (moderate to severe) or 6 (severe) according to Bernet et al. (1999).

2.8 Statistical analysis

Statistical analysis were generally conducted with SigmaPlot 12.5 (Systat Software Inc., San Jose, USA), with exception of mutagenicity determination by the rate of revertants (see below), which was computed with ToxRat 2.10 (ToxRat Solutions GmbH, Alsdorf, Germany). ANOVA on ranks (multiple comparison) followed by Dunn’s post hoc test (α=0.05) was used to determine significant differences in the *in vitro* EROD induction by the sediment extracts, and the graded histopathological changes of liver and kidney, in comparison to the reference (BJX-R and BJX 2012, respectively). The same was applied for pairwise comparison of the Fulton condition factors for fish from all sites. Mann-Whitney Rank Sum Test (α=0.05) was conducted to compute significant differences in hepatic EROD and GST activities, as well as micronucleus formation of the samples in comparison to the reference (BJX 2012). In order to identify significant correlations between chemical analysis, *in vitro* EROD induction assay and the *in vivo* FET, as well as between the biliary 1-OHPr content and hepatic EROD and GST activities the Spearman Rank Order Correlation was applied (α=0.05).

To determine mutagenicity by the rate of revertants Shapiro-Wilk’s test on normal distribution and Levene’s test on variance homogeneity (with residuals) were performed as pretesting sequences. If data showed normal distribution and variance homogeneity William’s multiple sequential t-test (α=0.05) was used to determine NOEC values. The results of these tests allow for the use of the parametric William’s test without further transformation. If data showed normal distribution but variances were heterogeneous, Welch-t test with Bonferroni-Holm adjustment was performed.

Deviations of the sample values from control are classified as significant with a p-value smaller than a significance level of 5 % (*) or 1% (**) and as highly significant with p-value smaller than a significance level of 0.1 % (***).
Part 3

PAH Metabolism in Immune Organs

This chapter has been published as part of an article in a peer-reviewed journal:

3 PAH metabolism in immune organs

3.1 Chemicals

BaP and all other chemicals were purchased from Sigma-Aldrich GmbH (Buchs, Switzerland). BaP metabolites (benzo[a]pyrene-trans-7,8-dihydropyrrole, benzo[a]pyrene-trans-4,5-dihydropyrrole, benzo[a]pyrene-trans-9,10-dihydropyrrole, 3-hydroxybenzo[a]pyrene and 9-hydroxybenzo[a]pyrene) were purchased from the NCI Chemical Carcinogen Reference Standards Repository (Kansas City, Missouri, USA). Acetonitrile, methanol, ethyl acetate and acetone were purchased from Carl Roth GmbH & Co. KG (Arlesheim, Switzerland). MS-222 was purchased from Sandoz LTD (Basel, Switzerland).

3.2 Experimental animals

Rainbow trout (average weight: 300 g) were obtained from a local hatchery (cantonal fish farm Kandersteg, Switzerland). Experiments were carried out according to the Swiss Animal Welfare regulations. Fishes had an average standard length of 280 ± 33 mm and an average weight of 260 ± 85 g. Classification of maturity stages was performed on histological sections for female and male trout according to Körner et al. (2007). Maturity was stage 1 (immature oocytes) to 2 (presence of cortico-alveolar oocytes) in female trout and at stage 2 (all maturity stages of spermatocytes equally present) to 3 (mature spermatocyte stages are dominant) in male trout. One animal had undifferentiated gonads. During the experiments, each individual fish was kept in a separate 120 L glass aquarium supplied with tap water in a flow-through system. Tanks were aerated and water temperature during experiments was 15°C ± 1°C.

3.3 Experimental treatments and sampling

Fishes were treated by intraperitoneal injection either with corn oil (control) or with 15 mg/kg BaP in corn oil (BaP exposure). The selected BaP concentration is in the lower range of dosing concentrations that are found in literature (2-200 mg/kg). During the exposure, trout were fasted. Five days after dosing, trout were sacrificed by an overdose of MS-222, followed by a blow to the head. The tissues collected from these fish were used for the following investigations:

- Bile fluid was collected from five control and five BaP-treated fish and analyzed for BaP metabolites in order to verify that BaP injection successfully induced BaP metabolism and excretion.
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- Liver (n=5) and immune tissues (n=3) of control and BaP-exposed fish were collected and analyzed for patterns of BaP metabolites, in order to estimate tissue-specific BaP metabolism \textit{in vivo}.

- Microsomes were prepared from liver and immune tissues of control and BaP-exposed fish, and were incubated \textit{in vitro} with 7-ethoxyresorufin in order to analyze EROD activity (n=3).

- Microsomes were prepared from liver and immune tissues of control and BaP-exposed fish, and were incubated \textit{in vitro} with BaP in order to (i) compare BaP metabolite patterns and (ii) BaP metabolic rate between the organs (liver: \( n=1 \), immune tissues: \( n=2 \)).

Tissue and bile samples were stored at -80°C until further processing. Microsomes were prepared immediately from freshly dissected tissues. Preparation was done according to Harris et al. (2009), and aliquots were stored frozen at -80°C until use for experiments. Microsomal protein content was determined according to the method of Bradford (1976). In addition to liver and immune organs, gonadal tissue was fixed in formaldehyde 4% for histological determination of sex and maturity.

3.4 Bile analysis

Aliquots of bile fluid of five fish were used to determine the sum of BaP metabolites. Determination of the sum of BaP metabolites in the bile was achieved by the fluorescence method of Aas et al. (2000) and Lin et al. (1996), with the excitation/emission wavelength pair of 380/430 nm. Bile fluid was diluted 1:2000 with double distilled water, and 200 \( \mu L \) of the diluted bile were placed into individual wells of an opaque 96 well plate and measured for fluorescence using a fluorescence plate reader (EnSpire 2300 Multilabel Reader, Perkin Elmer AG, Schwerzenbach, Switzerland). Fluorescence values were expressed as arbitrary fluorescence units (fu) and data is expressed as mean fu value \( \pm \) SD.

Other aliquots of the bile fluid were used to determine individual BaP metabolites. To this end, bile samples were extracted using a protocol adapted from Willett et al. (2000). To 50 \( \mu L \) bile, 1250 \( \mu L \) sodium acetate buffer were added containing 1000 U \( \beta \)-glucuronidase and 19 U arylsulfatase (from \textit{Helix pomatia}, Sigma-Aldrich GmbH, Buchs, Switzerland). Mixtures were incubated overnight at 40°C for deconjugation. Afterwards, they were extracted as described.
by Willett et al. (2000) and individual metabolites were analyzed using HPLC (see below). Data is expressed as mean ng substance per μL bile ± SD.

3.5 Tissue preparation and extraction for HPLC analysis

Tissue samples were extracted using a modified method of Ramesh et al. (2001) and Willett et al. (2000). Livers were collected from five animals each of the control and of the BaP-exposed group, spleen and head kidneys from three trout per group. Livers were perfused with PBS to remove the blood. Organ samples (1 g) from individual animals were homogenized in two volumes of sodium acetate buffer (0.4 M, pH 5.0). Metabolite conjugates in the homogenates were deconjugated enzymatically by incubation at 40°C overnight with β-glucuronidase (1000 U) and aryl-sulfatase (19 U, both from Helix pomatia, Sigma-Aldrich, Buchs, Switzerland). Subsequently, to precipitate proteins, sodium dodecyl sulfate (1 %) was added and mixed for 1 min. Deconjugation products were extracted twice by liquid-liquid extraction with 2 volumes ethyl acetate:acetone (2:1). Extraction tubes were mixed (1 min) and centrifuged (5000 × g, 20 min, 10°C). Organic fractions were combined and dried down under a stream of nitrogen. The residue was dissolved in 400 μL of acetonitrile. The aqueous phase was adjusted to pH 1 with 1 N HCl to deconjugate glutathionated metabolites (Yu et al. 1995, Beyer et al. 2010). This step was followed by a 3-step liquid-liquid-extraction (2 volumes ethyl acetate:acetone (2:1), 2 × 2 volumes ethyl acetate). The organic phase was dried down under nitrogen and dissolved in 200 μL acetonitrile. Acetonitrile fractions were combined and analyzed using HPLC and fluorescence detection. Values are given as mean ng substance per mg tissue ± SD.

3.6 Microsomal incubations

Microsomal incubations and extraction for metabolism studies were performed as described previously - Harris et al. (2009), for microsomal incubation; Stuchal et al. (2006), for extraction. The in vitro metabolism assay was performed with microsomal protein concentrations of 0.5 mg/mL and substrate concentration of 5 μM BaP. Control assays included incubations that were immediately stopped after BaP exposure and incubations without NADPH. Liver microsomes were incubated for 2 h, spleen and head kidney microsomes for 3 h, respectively, at 21°C. The extended incubation period for microsomes from immune tissues was selected to obtain sufficient amounts of metabolites. The reactions were stopped by addition of 5 mL of ethyl acetate:acetone (2:1); afterwards they were extracted (Stuchal et al. 2006, Harris et al. 2009). The organic phase including metabolites was dried down under nitrogen, dissolved in
acetonitrile and analyzed in the HPLC. Data is expressed as mean ng substance per mg protein per h ± SD for BaP-treated and control microsomes from head kidney and spleen (n = 2, respectively). One control and one BaP-treated fish were sampled for liver microsomes.

3.7 HPLC analysis of extracts from in vivo tissues and from in vitro microsomal incubations

Samples were subjected to HPLC analysis for detection of BaP and BaP metabolites. Given the differences between liver, spleen and head kidney in tissue structure, composition, and blood perfusion, we placed emphasis on comparing relative capacities for metabolite formation in liver and immune tissues. Our analytical method was based on the methods of Beyer et al. (2010) and Ramesh et al. (2001), determining the concentration of the target analyte by comparing retention times and peak areas of samples with that of standards. A dilution curve was prepared for each metabolite standard and BaP at concentrations between 0.1 and 8 ng/μL. This standard curve was linear for each substance. The limit of detection was evaluated for each substance and was defined as the concentration which resulted in a peak approximately three times above the baseline. To identify BaP metabolites in tissue extracts and microsomal incubations, individual standards were added to the biological sample matrix of BaP-treated fish and the respective peaks were assigned by the resulting peak addition.

A Dionex HPLC system (Dionex P680 HPLC pump, ASI-100 automated sample injector, RF-2000 fluorescence detector, Dionex AG, Reinach, Switzerland) was used for sample analysis. Before injection, the extracts were centrifuged (10 min, 9300 × g, 10°C). Using an automated sampler, 25 to 150 μL were injected onto a C18 reversed phase column (Supelcosil LC-PAH C18, 150 × 4.6 mm, 5 μm, Sigma-Aldrich, Buchs, Switzerland). Separation of analytes was achieved at a flow rate of 1 mL/min as follows: 30:70 acetonitrile: H2O was held for 5 min, followed by a linear gradient to 85:15 acetonitrile: H2O in 35 min. This was held for another 10 min, before returning to 30:70 acetonitrile: H2O in 10 min. Fluorescence was monitored at excitation/emission wavelengths 320/430 nm.

3.8 EROD activity

Ethoxyresorufin-O-deethylase (EROD) activity was determined in head kidney, spleen and liver microsomes to characterize organ-specific monooxygenase activity. Microsomes from BaP-treated and control fish were analyzed. Enzyme kinetic analyses were performed in black 96 well plates with 250 μL reaction volume per well using a protocol adapted from Nakayama
et al. (2008). As substrate, 7-ethoxyresorufin was used (16 μM). Conversion of 7-ethoxyresorufin into fluorescent resorufin was measured immediately after addition of the microsomes at the excitation/emission wavelength pair 544/590 nm in a fluorescence plate reader (EnSpire 2300 Multilabel Reader, Perkin Elmer AG, Schwerzenbach, Switzerland). Resorufin formation was linear under conditions of the measurement. Each sample was analyzed at three different protein amounts: 2.5, 5 or 10 μL of undiluted microsomes (the microsomal protein concentration was approximately 2 mg/mL). Microsomes from head kidney, spleen and liver from three control or BaP-treated animals were used. Data is expressed as mean pmol resorufin per minute per mg protein ± SD. For reaction rate analysis, GraphPad Prism software Version 5.02 (GraphPad Software Inc., San Diego, USA) was used.

3.9 Statistics

Differences between treatment groups were analyzed using Student’s t test. Metabolite levels in treatment groups were analyzed for significant differences by one-way analysis of variance (ANOVA), followed by a Tukey-Kramer Multiple Comparison test. All statistical analysis was performed using GraphPad Prism software (Version 5.02, GraphPad Software Inc., San Diego, USA).
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Chapter III

Results & Discussion
-Part 1-

The ecotoxicological status of the Yangtze River

This chapter has been published as part of an article in a peer-reviewed journal:

Chapter III – Results & Discussion

1 The ecotoxicological status of the Yangtze River

1.1 Abstract

The Yangtze River has been a source of life and prosperity for the Chinese people for centuries and is habitat for a remarkable variety of aquatic species. But the river suffers from huge amounts of urban sewage, agricultural effluents and industrial wastewater as well as ship navigation wastes along its course. With respect to the vast amounts of water and sediments discharged by the Yangtze River it is reasonable to ask whether the pollution problem may be solved by simple dilution. This chapter reviews the past two decades of published research on organic pollutants in the Yangtze River and several adjacent water bodies connected to the mainstream, according to the triad approach as a holistic assessment method. Organic pollutant levels and potential effects of water and sediments on wildlife and humans, measured in vitro, in vivo and in situ, were critically reviewed. The contamination with organic pollutants, including PAHs, PCBs, OCPs, PCDDs/DFs, PBDEs, PFCs and others, of water and sediment along the river was described. Especially Wuhan section and the Yangtze Estuary exhibited stronger pollution than other sections. Bioassays, displaying predominantly the endpoints mutagenicity and endocrine disruption, applied at sediments, drinking and surface water indicated a potential health risk in several areas. Aquatic organisms exhibited detectable concentrations of toxic compounds like PCBs, OCPs, PBDEs and PFCs. Genotoxic effects could also be assessed in situ in fish. To summarize, it can be stated that dilution reduces the ecotoxicological risk in the Yangtze River, but does not eliminate it. Keeping in mind an approximately fourteen times greater water discharge compared to the major European river Rhine the absolute pollution mass transfer of the Yangtze River is of severe concern for the environmental quality of its estuary and the East China Sea. Based on the review further research needs have been identified.
1.2 Pollutant levels in water and sediment

The water quality in the Yangtze River has deteriorated since the 1990s due to the anthropogenic activities in China (Yang et al. 2008). To ensure the health of aquatic biota and human beings in surrounding areas, the Chinese scientists and international communities pay close attention to the pollution status of the Yangtze River. For instance, the YVWEMC investigated micro-organic pollutants in water, sediments, and fish at the mainstream of the Yangtze River in 1990s (Wang & Peng 2002). In order to study the water quality in the Yangtze River, a sampling campaign was carried out by Swiss and Chinese scientists in 2006. They sampled hundreds of water and sediment samples from below the TGD to the river mouth at Shanghai, to determine major anions and cations, nitrogen and phosphorus, dissolved and particulate trace elements, and some organic pollutants (Müller et al. 2008). In addition, a number of studies were conducted to characterize the pollution of different environmental matrices with organic contaminants such as OCPs (Liu et al. 2011a) and perfluorinated compounds (PFCs) in water (So et al. 2007), as well as perfluorooctane sulfonates (PFOS) in fish (Greenpeace 2010) have been conducted along the river. Several pollutants, such as PAHs, PCBs and OCPs, in the Yangtze River have been studied for a long time with sufficient information at some reaches. Apart from those “typical pollutants”, new emerging contaminants like PBDEs and PFCs have recently attracted much attention in China (Wang et al. 2010a), as well as their occurrence in the Yangtze River (So et al. 2007, Xian et al. 2008). This part describes and compares the available data on the occurrence of the predominant organic pollutants, which were reported in water and sediments of the Yangtze River during the last two decades: PAHs, PCBs, OCPs, PCDDs/DFs, PBDEs, PFCs, in addition to phthalic acid esters (PAEs), nonylphenol (NP) and bisphenol A (BPA).

1.2.1 Polycyclic aromatic hydrocarbons

PAHs are a class of compounds that consist of three or more fused benzene rings with only carbon and hydrogen atoms (ATSDR 2009). PAHs are usually produced by incomplete combustion or high-pressure processes, and mainly originate from natural (oil seepage, biomass burning, volcanic eruptions and diagenesis) and anthropogenic sources (fossil fuel combustion and industrial processes) (Yunker et al. 2002, Wang et al. 2007). PAHs mostly act as carcinogens and mutagens, e.g., certain PAH metabolites are genotoxic and may interact with DNA, causing malignancies and heritable genetic damage in humans (ATSDR 2009). The 16 PAHs, chosen as priority pollutants by the U.S. Federal Water Pollution Control Act (1972) and the US Clean Water Act (1977) (Huang et al. 2003, Tobiszewski & Namieśnik 2012), have
been more commonly studied, among which, benzo[\(a\)]pyrene (BaP) is used as an environmental indicator for PAHs (ATSDR 2009). In the investigation of the YVVEMC PAHs were one of the main organic pollutants in the Yangtze River during the 1990s, while Chongqing and Nanjing section presented more serious PAH pollution than other sections (Wang & Peng 2002). The PAHs in water and sediments of the Yangtze River were summarized in Fig. III-1.1.

**Water:** The highest concentration of PAHs was reported at Panzhihua section in the Jinsha River, ranging from \(2.1 \times 10^4\) to \(3.83 \times 10^5\) ng/L with an average concentration of \(1.06 \times 10^5\) ng/L (Huang et al. 2003). The lowest concentration of PAHs was observed at the TGR (14-97 ng/L) (Wang et al. 2009). Higher concentrations of PAHs in the Yangtze River were detected at the Wuhan section (mainstream: 322-6,235 ng/L; tributaries: 242-1,379 ng/L) (Feng et al. 2007a) and Jiangsu section (40-3,345 ng/L) (He et al. 2011). In comparison to other rivers in China such as the Yellow River (97-477 ng/L) (Zhang 2010) and the Tai Lake (469 ng/L) (Zhang et al. 2012a), the concentration of PAHs at Wuhan section was higher in the Yangtze River. Concentrations in the South Branch of the Yangtze Estuary ranged from 478 to 5,027 ng/L (mean: 1,727 ng/L) in high water period (August) and 972 to 6,273 ng/L (mean: 1,988 ng/L) in low water period (February) (Ou et al. 2009). The level of PAHs in the Yangtze Estuary share the similar level with the Pearl Estuary (987-2,879 ng/L, mean: 1,796 ng/L) (Luo et al. 2004), while they are lower than that at Macao harbor (944-6,655 ng/L, mean: 4,124 ng/L) (Luo et al. 2004). The PAH pollution status has significantly increased in the past decades, in comparison to PAH levels along the river in 1990s (n.d.-135 ng/L, mean: 22 ng/L) (Wang & Peng 2002). So far, no regulatory limit has been imposed on any PAHs, except BaP (2.8 ng/L), for the surface water in China (Ministry of Environmental Protection - China 2002). Fig. III-1.1a presents the concentrations of total PAHs and BaP reported at each section. It is, however, interesting that BaP was not detected in any sampling station in the Jinsha River (Huang et al. 2003), while the predominance of benzo\([k]\)fluoranthene (BkF) and indeno\([1,2,3-c,d]\)pyrene (IcdP) was clearly observed in this area (Huang et al. 2003). This is quite different from the results of other sections in the Yangtze River reported in the literature. The concentrations of BkF (16-296 \(\mu\)g/L) and IcdP (4-83 \(\mu\)g/L) in the Jinsha River exceeded the criteria recommended by the European Union (sum of BkF and benzo\([b]\)fluoranthene: 30 ng/L, and sum of benzo\([g,h,i]\)perylene and IcdP: 2 ng/L) (EWFD Directive 2008/105/EC). In addition, the maximum concentrations of BaP in Wuhan section (n.d.-214 ng/L) (Feng et al. 2007a) and Jiangsu section (n.d.-768 ng/L, mean: 256 ng/L) (He et al. 2011) exceeded both the Chinese regulatory limit (2.8 ng/L) and the European Directive for surface water (50 ng/L) (EWFD
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Directive 2008/105/EC). The highest concentration of BaP was observed at Nanjing section, ranging from n.d. to 768 ng/L (mean: 256 ng/L). The pollution was attributed to some heavy chemical industry in the districts of Nanjing (He et al. 2011).

![Bar chart showing concentrations of PAHs and BaP in water and sediments of the Yangtze River.](image)

**Sediment**: Compared to the Lower Reaches, especially the Yangtze Estuary, sediments in the Upper Reaches of the Yangtze River are not well investigated (Fig. III-1.1b). The Upper Reaches of the river contain few sediments due to the rapid flow and special local geographical features (Huang et al. 2003). According to the present studies at Chongqing section (257-723 ng/g, mean: 359 ng/g) (Tang et al. 2011) and the Jialing River (132-349 ng/g, mean: 240 ng/g) (Tang et al. 2011), the levels of PAHs in the Upper Reaches were lower than those in Middle and Lower Reaches. The concentration of PAHs in sediments at Wuhan section showed a higher level, ranging from 303 to 3,995 ng/g (mean: 2,032 ng/g) in the mainstream and 4,121 to 4,262 ng/g in the tributaries (Feng et al. 2007a). The highest concentration of core sediments in the Yangtze Estuary (11,740 ng/g) was found near one sewage discharge point of Shanghai (Liu et al. 2000), in which sewage discharges were a significant input source. However, in comparison to the Yellow Estuary (11-252 ng/g, mean: 91 ng/g) (Hui et al. 2009a), the total PAH levels of the Yangtze Estuary in surface sediments (263-6,372 ng/g, mean: 1,662 ng/g) was significantly higher (Liu et al. 2001), but was lower in other urban areas in China, such as the Pearl Estuary (323-14,812 ng/g) (Mai et al. 2002) or elsewhere in the world, like San Francisco Bay (2,653-27,680 ng/g) (Pereira et al. 1996).

There have not yet been any official related criteria or standards to evaluate biological effects of Total PAHs in sediments. Two guideline values, “effect range-low (ERL)” and “effect range-medium (ERM)”, developed by Long et al. (1995), were commonly used to assess the adverse biological effects of contaminants in sediments. The assessment criteria are as follows: concentration < ERL: biological effects rarely occur; ERL ≤ concentration < ERM: biological effects occasionally occur; concentration ≥ ERM: biological effects frequently occur (Long et al. 1995). The effect range values for PAHs (ERL: 4,022 ng/g dry weight, ERM: 44,792 ng/g dry weight) demonstrate that the maximum concentrations of PAHs in the tributaries at Wuhan section (4,121-4,262 ng/g) (Feng et al. 2007a), one discharge point of Shanghai at the Yangtze Estuary (11,740 ng/g) (Liu et al. 2000), and the Yangtze Estuary (6,372 ng/g) (Liu et al. 2001) exceeded the ERL value, but were significantly lower than ERM value. It indicates that the PAHs may cause toxic effects. This is in accordance to a guidance proposal for the assessment of sediment quality presented by Ahlf et al. (2002), which gives a quality goal of 1,000-4,000 ng/g for EPA-PAHs in sediments. Values that exceed this goal require further analysis or immediate action, like remediation and excavation of the contaminated sediments. However, based on the available studies and according to a classification by the International Commission for the Protection of the Rhine (ICPR 2009), which is based on the European Water Framework Directive (EWFD) (EWFD Directive 2000/60/EC), an integrated river basin management to

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improve the quality of European water bodies, all of the BaP levels in sediments were below a “relevant contamination” (> fourfold ICPR target value) (800-1,600 ng/g) (ICPR 2009).

**Sources:** PAHs diagnostic ratios, such as ANT/(ANT+PHE), FLA/(FLA+PYR), IcdP/(IcdP+BghiP) (Table II-3) and ΣLMW/ΣHMW (Total Low Molecular Weight: sum of two and three-ring PAHs; Total High Molecular Weight: sum of four and five ring PAHs), provide an important tool for the identification of the responsible pollution emission sources (Mai et al. 2002, Wang et al. 2009, Tobiszewski & Namieśnik 2012).

Likely due to the development of the coal chemical industry at Panzhihua section, Huang et al. (2003) discovered that five- and six- ring PAHs were abundant at most sampling stations in the Jinsha River, and serious PAH pollution could mainly be attributed to the waste discharge of some local coking plants and many coal chemical industries in that area (Huang et al. 2003). The largest steel-making center in Southwest China and the largest national titanium and vanadium producing center is located in Panzhihua city and is considered to be the most important input source of hydrocarbons in Panzhihua region. According to the molecular ratio of ANT/(ANT+PHE), BaA/(BaA+CHR) and FLA/(FLA+PYR), the combustion of coal, grass and wood, as well as wastewater runoff were the main sources of PAHs at Chongqing section (Wang et al. 2009). Meanwhile, with the construction of the TGD, the intensification of ship traffic and decreased water flow, which results in higher sedimentation rates of potentially contaminated dissolved organic matter, may elevate levels of PAHs near the dam. The PAH contamination of sediments at Wuhan section was mainly dominated by three-, four-, and five-ring PAHs. The ratios suggested that the contamination was mainly caused by combustion, including wood, coal and petroleum (Feng et al. 2007a). The PAH composition in the Yangtze Estuary, in which four- to six- ring PAHs were dominant, showed that they mainly derived from petroleum combustion, vehicle emission, and biomass combustion (mainly coal) in the near-shore area (Wang et al. 2012b). Whereas two- to three- ring PAHs were chiefly presented in the farther shore zone, originating from petroleum combustion of shipping processes and shore side discharges (Wang et al. 2012b). Chongming Island, the largest alluvial island in the world, has been rapidly developing its town industry, agriculture, tourism and shipping (Li et al. 2009). The PAH ratios of ANT/(ANT+PHE), FLA/(FLA+PYR) and IcdP/(IcdP+BghiP) indicated that the sources of this site were a mixture of petroleum combustion, sewage, biomass and coal combustion. But the island is also influenced from the outside. Additional sources were particle bound PAHs discharged into the estuary from upstream sources, which accumulated around the
island. Also, atmospherically transported contaminants from Shanghai played an important role for the PAH input into the Yangtze Estuary (Feng et al. 2006).

1.2.2 Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs), which are composed of 209 possible congeners, are “a subset of chlorinated hydrocarbons” (UNEP 1999). Exposure to different levels of PCBs can lead to acute toxicity like skin rashes, itching, burning, eye irritation, immune system disorders, chronic effects including liver damage, reproductive and developmental defects, and possibly cancer (UNEP 1999, ATSDR 2000). Because of these characteristics, PCBs were designated as typical POPs on Stockholm Convention in 2001. There are two main input sources of PCBs in China: one was the commercial production of PCBs (approximately $10^4$ t, including $9 \times 10^3$ t trichlorobiphenyl mixture and 1,000 tons pentachlorobiphenyl mixture) from 1965 to 1974, and the other is the large quantity of imported PCBs transformers accompanied by other electrical equipment since 1970s (Xing et al. 2005, Bao et al. 2012). The PCBs usage in eastern China, including most of the Lower Yangtze Reaches, was reported to account for 45% of the whole national PCB contamination, with an average gross use density of 2.9 kg/km² (Xing et al. 2005). PCBs were reported to be the major organic pollutants of the Yangtze River in the investigation of YVWEMC in 1990s (Wang & Peng 2002).

**Water**: The total concentrations of PCBs in the Yangtze River ranged from undetectable levels to 44 ng/L (Chen et al. 2008, He et al. 2011). **Fig. III-1.2a** presents the concentrations of total PCBs in water of the Yangtze River. The levels of PCBs (0.01-1 ng/L) at TGR in 2008 (Wang et al. 2009) present an increase in comparison to the concentrations reported in 2004 (n.d. to 0.01 ng/L, mean: 0.002 ng/L) (Chen et al. 2008). The highest level of PCBs in water was found at the outlet of Nanjing city, ranging from 0.2 to 44 ng/L with an average concentration of 11 ng/L (He et al. 2011). Compared to the data from 1999 at Nanjing section (up to 3 ng/L) (Sun et al. 2002), it is found that the concentrations of PCBs increased significantly in that area. Taking the quality standards of surface water in China (20 ng/L) into account (Ministry of Environmental Protection - China 2002), attention needs to be paid to the risk posed by PCBs at Nanjing section to human health as well as to the surrounding environment. Ye and coworkers attributed the existence of PCBs in Nanjing section mainly to discharged wastewater from a hormone-producing plant (Ye et al. 2009). The concentration of PCBs in the Yangtze Estuary ranged from 1 to 17 ng/L (Zhang et al. 2011). Compared to the levels of PCBs in the Pearl Estuary (0.1-2 ng/L) (Guan et al. 2009), this concentration was significantly higher. Concentrations of PCBs in the Yangtze River decreased significantly in comparison to the
levels in 1990s (n.d. to $53.5 \times 10^3$ ng/L, mean: 869 ng/L) (Wang & Peng 2002). PCB levels increased in most areas of China during the 1980s and 1990s, what can be attributed to improper disposal and leakage from PCB-containing equipment (Xing et al. 2005).

**Sediment:** Zhao et al. (2013) recently reported the levels of PCBs in sediments at TGR, ranging from 0.5 ng/g to 4 ng/g. Compared to other reaches of the Yangtze River, PCB levels in TGR are extremely low. The concentrations of PCBs in sediments at Wuhan section ranged from 1 to 45 ng/g (mean: 9 ng/g) (Yang et al. 2009b), which were higher than those found in the upper and middle reaches of the Yellow River (up to 6 ng/g) (He et al. 2006). The sampling site was close to discharge points of wastewater treatment plants and various chemical companies in the tributaries. In comparison to urban areas in other parts of the world, such as the Elbe River (45-64 ng/g) (Kiersch et al. 2010), the Rhine River (1-32 ng/g) (Wölz et al. 2008), the Upper Danube River (n.d.-0.2 ng/g) (Keiter et al. 2008) and the Neckar River (1-14 ng/g) (Wölz et al. 2008), the detected levels in Yangtze River were in the medium range. The concentration of PCBs in Yangtze Estuary ranged up to 51 ng/g (Cheng et al. 2006), which is far less than in the Pearl Estuary (10-486 ng/g) (Mai et al. 2002), and the Yellow Estuary (0.004-180 ng/g) (Hui et al. 2009b). The higher levels in sediment than in water can be attributed to the characteristics of PCBs which are less soluble in water and have high octanol-water partition coefficients, and thus can ultimately accumulate in bottom sediments due to their strong affinity to particulate matter.

The ERL and ERM guideline values have been frequently applied to assess the associated biological risks growing from PCB pollution in sediments. PCB levels above the ERL value (22.7 ng/g) suggest toxic effects on aquatic organisms, while a comparably high ERM value (180 ng/g) indicates a high possibility of detrimental effects (Long et al. 1995). All mean concentrations of PCBs in the Yangtze River were lower than the ERL values. However, the maximum concentrations of PCBs in the tributaries at Wuhan section (45 ng/g) (Yang et al. 2009b), Hangzhou Bay in Lower Yangtze Reaches (52 ng/g) (Cheng et al. 2006), and the South Branch of Yangtze Estuary (30 ng/g) (Hui et al. 2009b) exceeded the ERL limit (Fig. III-1.2b), suggesting that these levels of PCBs may cause biological impairments. However, according to a classification by ICPR (2009), the PCB levels were all below the “relevant contamination” level (> fourfold ICPR target value) ($\Sigma$PCBs$_7$, fourfold target value: 56-122 ng/L) (ICPR 2009).
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Sources: There are no natural sources of PCBs and the major input into the aquatic environment is via atmospheric deposition, runoff from the land, and transport through food chains (Yang et al. 2009b). Trichlorobiphenyl mixtures were the predominant PCB congeners observed in sediment of TGR, suggesting that transformer or capacitor oils were the main sources, which have been carried via runoff into the water body. Furthermore, atmospheric depositions also contributed large loads of PCBs at TGR. PCBs at Wuhan section originated mostly from land runoff during floods and heavy rains (Yang et al. 2009b), in addition to wastewater from municipal and industrial sources, atmospheric deposition, and upstream sources (Morrison et al. 2002). The conclusion is in agreement with the study by Yang et al. (2012b), who claimed that agricultural soils might be a crucial input source for PCBs in the sediments of the Yangtze Estuary. Moreover, the pattern of PCBs in core sediment profiles of the Yangtze Estuary and the adjacent East China Sea demonstrated an increasing pollution level from 1980s to 2000. This can be explained by PCB-containing equipment imported from industrialized countries over the last two decades. A decreasing tendency could be shown after the Chinese Government prohibited the import of electronic waste (e-waste) at the beginning of 2000s (Yang et al. 2012b).

1.2.3 Organochlorine pesticides

Well-known organochlorine pesticides (OCPs) comprise hexachlorocyclohexanes (HCHs, sum of α-,β-,γ-,δ-HCH), dichlorodiphenyldichloroethane (DDT) and its primary metabolites (the sum of o,p’-DDT, p,p’-DDT, dichlorodiphenyldichloroethane (DDD), and dichlorodiphenyldichloroethylene (DDE); designated as DDTs), aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex and toxaphenes (Bodo 1996, Vijgen et al. 2011, Bao et al. 2012), among which DDT and HCHs have been widely used as pesticides to control insects on agricultural crops worldwide in the 1940s and the 1950s. DDT and its metabolites may increase the risks for breast cancer and play a role in endocrine disruptions (Turusov et al. 2002, Cohn et al. 2007, Wetterauer et al. 2012). The toxicological effects of various isomers of HCHs can be as diverse as renal and liver failures, blood circulation disorders, imbalance in biochemical homeostasis, and reproductive defects in laboratory animals (Bodo 1996, Willett et al. 1998). To protect the health of living organisms in the environment, DDT was subsequently limited for malaria control under the Stockholm Convention in 2001 (UNEP 2001); while HCHs (α-, β-, γ- HCH) were added as three of nine new POPs in the Stockholm Convention in 2009 (UNEP 2009). China banned the production of DDT and HCHs in 1983 and 1984, while large amounts of DDT (0.4 million tons) and HCHs (4.9 million tons) were
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produced during the 1950s to early 1980s (Bao et al. 2012). Though the residues of OCPs in the environment have considerably declined in the past decades (Wang & Peng 2002), the levels of OCPs can still be detected in various environmental matrixes along the Yangtze River. DDTs and HCHs have been observed in Tuotuo River (the origin of the Yangtze River), even though OCPs have never been used in that area (Liu et al. 2011a). A recent study reported the bioaccumulation of DDTs and HCHs in sediment-dwelling animals like mollusks and crabs in the Yangtze River (Yang et al. 2006), while another recent study indicated new input sources of HCHs in the Wuhan area (Tang et al. 2007b), to which attention should be paid. Furthermore, Pentachlorophenol (PCP) has been recognized to be bioaccumulative and cause endocrine disrupting effects, mutagenicity and carcinogenicity (Seiler 1991, Vom Saal & Hughes 2005), and is often contaminated with dioxins and furans (Zheng et al. 2012). Figure III-1.3 summarizes the occurrence of OCPs in the Yangtze River.

Water: Currently available data suggest that the Yangtze River from headstream to estuary is contaminated by OCPs (Fig. III-1.3a). To investigate the levels of DDTs and HCHs in the Yangtze River, Liu et al. (2011a) sampled 37 natural water samples from Tibet (headstream) to the estuary along the Yangtze River. The total HCHs (sum of α-, β-, γ-, δ- HCH) were in the range of 0.1 to 14 ng/L (mean: 3 ng/L). The DDTs (sum of p,p'-DDT, o,p'-DDT, p,p'-DDE, p,p'-DDD) ranged up to 21 ng/L (mean: 4 ng/L). The total concentration of OCPs (sum of HCHs and DDTs) could be detected in a range of 0.1 to 27 ng/L (mean: 3 ng/L) (Liu et al. 2011a). Levels of OCPs were reported to be stable along the Yangtze River except some areas near industrial sites (Liu et al. 2011a). It is also interesting to find that OCP levels in Tuotuo River, which is the source of the Yangtze River, were similar to those of the Yangtze Delta (Liu et al. 2011a). The highest concentrations of HCHs (0.1-28 ng/L), DDTs (0.1-17 ng/L) and total OCPs (0.3-47 ng/L) were detected by Tang et al. (2008) in the tributaries at Wuhan section. These tributaries pass areas of intensive agricultural activities, where HCHs had been widely used (Wu et al. 1997, Tang et al. 2008). OCP levels of the Yangtze River were lower, compared to the concentrations measured in the Yellow River (3-109 ng/L) (Sun et al. 2009) and the Pearl River (3-41 ng/L) (Guan et al. 2009), and were much lower than those in Macao Harbor (25-68 ng/L) (Luo et al. 2004). None of the concentrations in the Yangtze River exceeded the quality standards of HCH (lindane) (2,000 ng/L) and DDTs (1,000 ng/L) in surface water recommended by the Ministry of Environmental Protection - China (2002), as well as quality standards in drinking water (DDTs; 1,000 ng/L, lindane: 2,000 ng/L) (Ministry of Health - China 2006). Taking the regulatory limit (DDTs: 25 ng/L) for drinking water set forth by the European Union (EWFD Directive 2008/105/EC) into consideration, the maximum
concentration of DDTs at Wuhan section are also below the limit (Fig. III-1.3a). Han et al. (2009) investigated PCP levels in water of the Yangtze River at Jiangsu section and found that the related PCP levels ranged from undetectable to 220 ng/L, which is below the regulatory value (400 ng/L) recommended by European Union (EWFD Directive 2008/105/EC). The levels of heptachlor epoxide along the Yangtze River were lower than the Chinese surface quality standards (200 ng/L) (Ministry of Environmental Protection - China 2002). However, the concentration of heptachlor epoxide in some areas was higher than the Criterion Continuous Concentration (CCC) (3.8 ng/L) recommended by the Environmental Protection Agency - USA (2009b) (USEPA). For instance, one high concentration of heptachlor epoxide (18 ng/L) was reported at Wuhan section, which was nearly 5 times higher than the USEPA guideline (Tang et al. 2008). The OCPs in surface water were generally within safe levels according to environmental standards in China. However, it does not mean that the potential adverse effects on ecosystems and human health should be neglected at particular sections.

**Sediment**: Levels of DDTs (2-16 ng/g) and HCHs (0.1-12 ng/g) in mainstream sediments at Wuhan section were lower than the levels in tributaries (DDTs: 1-36 ng/g; HCHs 0.2-21 ng/g) (Tang et al. 2007b). These results are in accordance with concentrations of DDTs and HCHs in the water of this area (Tang et al. 2008). The levels of DDTs in the tributaries at Nanjing section (4-32.6 ng/g) (An et al. 2006) and the South Branch of the Yangtze Estuary (1-33 ng/g) (Liu et al. 2008a) were higher than other sections. Lower concentrations were found in the East China Sea (DDTs: 0.1-6 ng/g, mean: 3 ng/g, HCHs: 0.1-2 ng/g, mean: 1 ng/g) (Yang et al. 2005), which are likely due to a very high dilution in the sea. The PCP levels in sediments of the Yangtze River at Wuhan section (1-2 ng/g, mean: 0.4 ng/g) were lower than those at Nanjing section (1-5 ng/g) (Xu et al. 2000b) and in Donghu Lake and Moshui Lake (n.d.-13 ng/g), which are also located at Wuhan section (Tang et al. 2007a). Based on current sediment quality criteria for DDTs in sediments, the concentrations of DDTs show that levels in all the sampling sections have exceeded ERL values (DDTs: 1.6 ng/g), but are lower than the ERM value (46.1 ng/g) (Fig. III-1.3b), suggesting that potential adverse biological risks exist in those areas. However, there is a lack of guidelines value about HCHs and PCP in sediments.
Fig. III-1.3. Maximum concentrations of OCPs, DDTs, HCHs in (A) water and (B) sediment of the Yangtze River. (A) OCPs, HCHs and DDTs in water compared to the regulatory limit (DDTs: 25 ng/L) for drinking water set forth by the European Union (EWFD Directive 2008/105/EC) (EC Directives). Three Gorges Reservoir (2008) (Wang et al. 2009); Sichuan section (2007) (Liu et al. 2011a); Wuhan section (2005) (Tang et al. 2008); Nanjing section (1998) (Xu et al. 2000a); Jiangsu section (2004-2005) (He et al. 2011); Jiangsu section (2007) (Liu et al. 2011a); (B) OCPs, HCHs, DDTs in sediments compared to ERL value (1.58 ng/g of DDTs) (Long et al. 1995). Wuhan section (MS) (2005) (Tang et al. 2007b); Wuhan section (TB) (2005) (Tang et al. 2007b); Nanjing section (MS) (1998) (Xu et al. 2000a); Nanjing section (TB) (2004) (An et al. 2006); Yangtze Estuary (2001) (Liu et al. 2003); Yangtze Estuary (SB) (2002) (Liu et al. 2008a); Yangtze Estuary (SB) (2006) (Liu et al. 2007); East China Sea (2002) (Yang et al. 2005). Numbers in brackets represent the sampling time; MS – mainstream; TB – tributary; SB – south branch; the locations are arranged in flow direction from left to right.
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\textbf{Sources:} Concentrations of OCPs have been detected in the Tuotuo River, though DDTs and HCHs have never been used in that area. Transport of OCPs to the origin of the river were explained by “cold condensation” and “the grasshopper-effect”, which means that OCPs are transported from the high temperature areas (Middle and Lower Yangtze Reaches) to the low temperature area (source region of the river) and precipitate there through cold condensation (Liu et al. 2011a).

Both lindane (“pure” \(\gamma\)-HCH) and technical HCH mixtures have been used in China from the 1950s to 1980s. The ratio of \(\alpha\)-HCH/\(\gamma\)-HCH can be used to diagnose the input sources of HCHs in the environment (Liu et al. 2011a, Bao et al. 2012). If the ratio varies between 0.2 to 1, this would mean that lindane is the typical input source in that area, while a ratio in the range of 4 to 7 would suggest that the HCHs in the environment originate from the usage of technical HCH mixtures (McConnell et al. 1993). The value of \(\alpha\)-HCH/\(\gamma\)-HCH ranged from 1.2 to 8.4 in the mainstream and 0.3 to 15.9 in the tributaries at Wuhan section, indicating that most of the HCHs were mainly derived from the technical HCH usage over the past decades. Lower ratios of \(\alpha\)-HCH/\(\gamma\)-HCH (<1) were observed in the tributaries at Wuhan section, suggesting that there could be possible input sources of lindane at those sections (Tang et al. 2008). Furthermore, high levels of \(\gamma\)-HCH in the Yangtze Estuary indicated that the HCHs derived from a continuous use of lindane rather than technical HCH usage in the Yangtze Delta area (Liu et al. 2008a).

DDT can be biodegraded to DDE under aerobic conditions and to DDD under anaerobic conditions. The diagnostic indexes of DDE/DDT, (DDE + DDD)/DDTs are used to differentiate the historical and recent inputs of DDTs (Metcalf 1973, Guo et al. 2009). According to the distribution of DDTs in core sediments studied by Yang et al. (2005), the decrease of DDTs concentration in the sediment occurred significantly after the official ban of DDTs in 1980s.

Besides, Wang and Peng (2002) found that DDTs levels in water of the Yangtze River in early 1990s were an order of magnitude lower than those in late 1990s. The ratio of (DDE+DDT)/DDTs in suspended particulate matter (SPM) varied from 0.4 to 0.96 (mean: 0.64) at Wuhan section, indicating that most of DDTs residues might be mainly aroused from the usage of technical DDTs over the past decades (Tang et al. 2008). Other studies in the Yangtze Estuary also confirmed this conclusion (Xu et al. 2000a, Liu et al. 2008a). In addition, the ratio of \(o,p'\)-DDT/ \(p,p'\)-DDT can be used as indicative indices for the input of technical DDT mixtures or dicofol-related sources (Guo et al. 2009). The “dicofol type DDT pollution” is defined as the DDT pollution caused by dicofol use and is characterized by higher \(o,p'\)-DDT/
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*p,p’*-DDT concentration ratio than that of technical DDT. The DDTs reported by Liu et al. (2008a) in SPM and Tang et al. (2007b), can be traced to the dicofol usage in some agricultural areas of the Middle and Lower Yangtze Reaches. PCP and its sodium salt (Na-PCP) are strong pesticides, which were used to control the spreading of schistosomiasis disease (Zheng et al. 2008). It is reported that Na-PCP with impurities of PCDDs/DFs was sprayed over the vast agricultural areas in the Middle and Lower Reaches of the Yangtze River since the 1960s. Zheng et al. (2012) assumed an increase of PCP contamination in environmental matrices, due to a growing use of Na-PCP to control the reemergence of schistosomiasis. The authors also emphasized that a double cancer risk from independent and joint effects of PCP and PCDD/DFs may raise further concerns (Zheng et al. 2012).

1.2.4 Polychlorinated dibenzo-p-dioxins/polychlorinated dibenzofurans

PCDDs/DFs represent groups of halogenated polycyclic aromatics, comprising 75 PCDD congeners and 135 PCDF congeners (Van den Berg et al. 1994, Zheng et al. 2008). There is no commercial use for PCDDs/DFs and they are unintentional byproducts of manufacturing processes. PCDDs/DFs can generate as impurities during the production of polychlorophenol under certain conditions (Zheng et al. 2008) or in combustion processes, e.g., solid waste burning in municipal incinerators, forest fires and volcanic eruptions (Van den Berg et al. 1994). PCDDs/PCDFs elicit a broad spectrum of biological and toxicological effects. Adverse effects on reproduction, development, endocrine functions and several types of dermal lesions have been observed in laboratory animals as well as wildlife species exposed to these compounds (Van den Berg et al. 1994, Luebke et al. 2001, Wen et al. 2008). Based on the contract of “Stockholm Convention on Persistent Organic Pollutants”, China and other countries in the world are taking measures to eliminate the possible sources of PCDDs/DFs (UNEP 2001). The sources of PCDDs/DFs in China were mainly derived from industrial wastes, production and usage of polychlorophenols, PCBs, chlorinated pesticides and triclosan which contained PCDDs/DFs as impurities (Zheng et al. 2008). Because of limited instrumentation and restricted trained personnel (UNEP 2002), the pollution status of PCDDs/DFs in water and sediments in China, especially the Yangtze River are rarely studied.

Water: Chen et al. (2008) reported the occurrences of PCDDs/DFs at TGR in the Upper Yangtze Reaches. The concentrations of total PCDDs/DFs in water ranged from 2 to 96 pg/L (mean: 12 pg/L), and the toxic equivalency (WHO-TEQ) of PCDDs/DFs in the TGR ranged from 0.001 to 0.3 pg/L (mean: 0.1 pg/L) (Chen et al. 2008). The concentration of 2,3,7,8-TCDD was undetectable, which is hard to compare with the tolerable limit of 2,3,7,8-TCDD (50 ×
$10^3$ pg/L) for surface and drinking water proposed by Di Domenico (1990). As there are no regulatory limits for surface water about PCDDs/DFs in China, this recommendation could be applied in the Yangtze River to improve analysis methods and risk control.

**Sediment**: Two studies reported about PCDDs/DFs in surface sediments of the Yangtze Estuary. Wen et al. (2008) detected the concentrations of total PCDDs/DFs and WHO-TEQs, ranging from 25 to 374 pg/g (mean: 170 pg/g), and from 0.4 to 1 pg/g, respectively. The latter were similar to those in the study of Sun et al. (2005), where WHO-TEQs of PCDDs/DFs ranged from 0.3 to 1 pg/g. The most abundant congener of PCDD in sediment was octachlorodibenzo-\(p\)-dioxin (OCDD) in the Yangtze Estuary (Sun et al. 2005, Wen et al. 2008), which accord with the PCDDs/DFs pattern in water of other places, like Jiangxi Province and Hubei Province in China (Zheng et al. 2008). The occurrence of PCDDs/DFs in TGR and Yangtze Estuary was attributed to the usage of Na-PCP in 1960s, which contains PCDDs/DFs as impurities, have been widely used in the middle and lower Yangtze River to control the spread of schistosomiasis disease (Zheng et al. 2008). The levels of PCDDs/DFs were lower than those in the Pearl River Estuary (Zhang et al. 2009a) and Mai Po Wetland (Müller et al. 2002), but marginally higher than that in the Yellow Estuary (Hui et al. 2009b) and Mondego Estuary (Spain) (Nunes et al. 2011). TEQ concentrations for the Yangtze River were below the value of 21.5 pg WHO-TEQ/g dw (1998) as the probable effect level on aquatic organisms suggested by Canadian Sediment Quality Guideline (CCME 2002).

1.2.5 **Emerging pollutants**

Concerning the organic contamination status of the Yangtze River the main attention was paid to the typical priority pollutants PAHs, PCBs and OCPs (including DDTs and HCHs). However, within the last two decades environmental risks of so called “emerging pollutants” raise increasing concern. So far, there is no international consensus with respect to a clear definition of “emerging pollutants”. Many constituents described as emerging pollutants are pharmaceuticals or personal care products (PPCPs) including endocrine disrupting compounds (EDCs) (Da Silva et al. 2012). Examples are polybrominated diphenyl ethers (PBDEs), perfluorinated compounds (PFCs) and phthalates (Environmental Protection Agency - USA 2008). This chapter summarizes and describes the detected levels of emerging pollutants (PBDEs, PFCs, PAEs, NP, BPA), which were obtained from the available articles in the scope of this review. These pollutants have been reported to be harmful to the environment and also been hypothesized to be harmful to human health. They mainly act as endocrine disruptors and

**Polybrominated diphenyl ethers:** PBDEs are a class of organobromine compounds that are used as flame-retardant additives. PBDEs have been used in a large variety of products, e.g., building materials, plastics, electrical appliances, television sets, computer circuit boards and casings (De Wit 2002, McDonald 2002). The environmental sources, metabolic relationships, and relative toxicities of PBDEs and their analogues, especially estrogenicity and androgenicity, effects on the thyroid hormone system has been reviewed by Wiseman et al. (2011). Long-time exposure to low concentrations of PBDEs can cause neurobehavioral deficits and might cause carcinogenicity (McDonald 2002). In addition, lower molecular weight congeners of PBDEs (tri- to hexa-BDEs) are highly bioaccumulative. Potential risks will come up when sensitive populations, like pregnant women, the developing fetuses or infants, are exposed to these substances (WHO/IPCS 1994, McDonald 2002). The industrial usages of some PBDEs are prohibited by the European Union and the United States of America. However, no such restrictions do apply in China to date (Mcpherson et al. 2004, Bao et al. 2012). In addition to the continuous usage of technical BDE mixtures, another main source of PBDEs in China is imported e-waste (Ni & Zeng 2009). A report set forth by Ni et al. (2010) estimated that about 70,600 tons of PBDEs were annually imported to China. The potential harm of PBDEs to human health and ecosystems, in addition to biotransformation of PBDEs to metabolites with even greater toxicities, has caused recently great concern (Wan et al. 2009, Wan et al. 2010, Wang et al. 2010a, Bao et al. 2012, Chen et al. 2012).

**Water:** It should be noted that there is no monitoring data of PBDEs in water of the Yangtze River. However, Federal Environmental Quality Guidelines (FEQGs) for PBDEs have been developed by Environment Canada to assess the ecological significance of PBDE levels (Environment Canada 2012). These guidelines can be utilized as references to control the presence of PBDEs in the Yangtze River.

**Sediment:** There is little information concerning the levels of PBDEs in sediments in the Upper Reaches of the Yangtze River except one recent publication reported by Zhao et al. (2013). Zhao and colleagues measured PBDEs at TGR, with total PBDEs (except BDE 209) and BDE 209 levels from n.d. to 0.15 ng/g and n.d. to 0.5 ng/g, respectively. Shen et al. (2006) investigated PBDE levels in sediments of the Yangtze River Delta (Yangtze Estuary, Hangzhou Bay and Qiantang River). 13 out of 32 sediment samples contained PBDEs, ranging from 2 to 4 ng/g (mean: 3 ng/g). These low levels of PBDEs were attributed to dilution effects. Huge
amounts of fresh water and associated suspended matter from the Upper Reaches diluted the organic contaminants in the Lower Reaches of the Yangtze River. However, higher concentrations were reported by Chen et al. (2006). The concentrations of $\Sigma_{12}$PBDEs (sum of 12 PBDEs congeners without BDE 209) and BDE 209 in sediment of the Yangtze Delta varied up to 1 ng/g (mean: 0.2 ng/g) and from 0.2 to 95 ng/g (mean: 13 ng/g), respectively. BDE 209 is a component of the commercial deca-BDE mixtures mostly used in China (Bao et al. 2012) and contributed 90-100% to the total PBDEs (Chen et al. 2006). In addition, the levels of lightly brominated congeners indicated that the atmospheric deposition was also a significant input source in the study area. Li et al. (2012c) studied PBDEs in sediments of the near-shore East China Sea, sampling from the Yangtze Estuary to about 1,000 km southward. The levels of BDE-209 and $\Sigma$PBDE7 ranged from 0.3 to 45 ng/g and up to 8 ng/g, respectively. Relatively higher PBDE concentrations were reported in the Yangtze Estuary and the south of Hangzhou Bay. PBDEs in the near-shore sediments of East China Sea were partly attributed to the input of the Yangtze River, because the areas with higher levels are the deposition center for fine-grained sediments (Li et al. 2012c). The maximum concentrations of BDE-209 in the Yangtze Estuary (Chen et al. 2006, Li et al. 2012c) exceeded the FEQGs of decaBDE as given by Environment Canada (19 ng/g) (Environment Canada 2012).

**Perfluorinated compounds:** PFCs are a category of organofluorine compounds, consisting of two well-studied compounds: perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). This group also contains compounds like perfluorononanoic acid (PFNA), perfluorobutanesulfonic acid (PFBS), perfluorooctanesulfonyl fluoride (POSF) and perfluorooctanesulfonamide (PFOSA). PFCs have been applied as surfactants and surface protectors in carpets, leather, paper, packaging, fabric, and upholstery as well as in or as aqueous film fire-fighting foams (AFFFs), mining and oil well surfactants, alkaline cleaners, floor polishes and photographic films (OECD 2002, Ahrens 2010). Stahl et al. (2011) reviewed the toxicodynamics of PFCs, such as acute toxicity, subacute and subchronic toxicities, chronic toxicity like immunotoxicity, carcinogenesis, genotoxicity and epigenetic effects, reproductive and developmental toxicities, neurotoxicity etc. Within the class of PFCs, PFOS and PFOA are generally considered as reference substances, and they can accumulate in serum and liver, causing hepatotoxicity (damage to the liver), forma adenomas (non-cancerous tumors) in liver and thyroid tissues, and decrease the serum cholesterol in rodents (OECD 2002, Suja et al. 2009, Giesy et al. 2010). Thus, PFOS, PFOA and its salts are proposed as a new class of POPs at the Stockholm Convention in May 2009 (Wang et al. 2010a, Vierke et al. 2012). Due to the potential negative effects of PFCs on human health, the United States of America and the
European Union formed a series of directives to prohibit the production of PFCs since 2000 (Ahrens 2010, Cai et al. 2012a). For instance, a major American Company, was once the most important global producer of PFCs, especially PFOS in 1940s to 1990s, but phased out its production in 2002 under the PFC reduce program launched by the USEPA (Ahrens 2010, Wang et al. 2010a). The major production of PFOS was shifted from North America and Europe primarily to China in the last decades (Cai et al. 2012a). The production of PFOS increased from less than 50 tons in 2003 to 247 tons in 2006 (Bao et al. 2010, Wang et al. 2010a). There were twelve PFC manufactures in China, according to the authors four have stopped the production and eight of them still produced. Three of those eight were located in Hubei Province, two in Fujian and the others in Beijing, Shanghai and Wuhan (Wang et al. 2010a). Since the beginning of 2000s Chinese scientists began to collect information of PFCs in the Yangtze River (Jin et al. 2006).

**Water:** Jin et al. (2006) have measured PFOS and PFOA in the Yangtze River at TGR and Wuhan section. They reported high levels of PFOS and PFOA in TGR, of which the maximum concentrations reached to 38 ng/L and 298 ng/L, respectively. The concentrations of PFOA upstream of Chongqing (0.2-0.4 ng/L) were far lower than downstream of Chongqing to Yichang (2-298 ng/L). High levels were detected at Xituo section (PFOA: 111 ng/L) and Fengdu section (PFOA: 298 ng/L). This indicated that input sources were located around these areas (Jin et al. 2006). However, the low average concentration at TGR can be explained by the dilution with enormous amounts of water. High concentrations of PFOA were also detected at Wuhan section (298 ng/L) (Jin et al. 2009) and Shanghai (260 ng/L) (So et al. 2007). This is in accordance with locations of PFC factories (Wang et al. 2010a). The highest concentration of PFOS (144 ng/L) was found in the South Branch of the Yangtze Estuary (Pan & You 2010). The sampling site is located at Baozhen Port, which serves as a freight terminal and passenger wharf. Significant amounts of domestic sewage, which have been reported to contain high levels of PFOS, were also considered as important sources (Pan & You 2010). PFC pollution in two interior lakes of the Yangtze River (Dian Lake and Tai Lake) has been recently reported (Yang et al. 2011, Zhang et al. 2012b). The PFC contamination in Tai Lake was different to other studies, with PFOS being the most abundant compound. This indicates a different origin compared to Chongqing, Wuhan and Shanghai. The authors attributed this to the production of paints, plastic pipes and plastic anticorrosion products, containing fluorinated chemicals, in Wuxi city (Yang et al. 2011). In 2009, the USEPA set short-term provisional health advisory values for PFOA and PFOS of 400 ng/L and 200 ng/L, respectively. According to the available
data, the levels of PFOA and PFOS in the Yangtze River are still below the USEPA guidelines (Environmental Protection Agency - USA 2009a).

**Sediment:** No research has been performed on PFCs in sediments of the Yangtze River except in the Yangtze Estuary. Li et al. (2010) reported a maximum concentration of PFOA (203 ng/g) in sediment from Huangpu River, which exceeded PFOA levels from many other studies (Bao et al. 2010, Pan & You 2010). Concentrations of PFOS were even higher in the South Branch of the Yangtze Estuary (73-537 ng/g) (Bao et al. 2010, Li et al. 2010). The sampling location was situated in the near shore region of Chongming Island. Correlation analysis between salinity and distribution coefficient of PFOS indicated that the affinity of PFOS to sediment was higher during salt intrusion. This means that PFOS can be carried from upstream sources for a long distance and will accumulate in the sediments of the Estuary, due to the dramatic change in salinity (Pan & You 2010). In comparison to other water bodies in China, like the Pearl River (Bao et al. 2010), Liao River (Yang et al. 2011), Tai Lake (Yang et al. 2011) and Dianchi Lake (Zhang et al. 2012b), the Yangtze River was more severely polluted with PFCs.

**Phthalic acid esters:** Phthalates are used as plasticizers in polyvinyl chloride (PVC) plastics, which are applied in the production of electrical cords, films, glues, paints, ink, varnishes, coatings, adhesives, cosmetics, pesticides, repellents, dielectric media (Jarosova 2006). In recent years toxicological concerns of PAEs were raised with regard to their potential endocrine disrupting potencies (Heudorf et al. 2007). Shi et al. (2012) studied thyroid hormone (TH) disrupting activities associated with phthalate esters in water of the Yangtze River Delta. The results indicated that di-n-butyl phthalate (DBP) was the primary TH receptor (TR) antagonist in water sources in the Yangtze River Delta, followed by di (2-ethylhexyl) phthalate (DEHP), di-n-octyl-phthalate (DnOP), di-isononyl phthalate (DiNP). This is in accordance with reports that describe DBP and DEHP as the predominant PAEs at the following sections. The concentrations of DBP and DEHP in Chongqing section (DBP: n.d.-42 μg/L, DEHP: n.d.-12 μg/L) (Luo et al. 2009), Wuhan section (DBP: n.d.-35 μg/L, DEHP: 4-54 μg/L) (Wang et al. 2008) and Yangtze Delta (DBP: n.d.-7 μg/L, DEHP: 4-28 μg/L) (Zhang et al. 2012a) all exceeded the surface water quality standard of China (DBP: 3 μg/L, DEHP: 8 μg/L) (Ministry of Environmental Protection - China 2002) as well as the regulatory limit of European Union (DEHP: 1.3 μg/L) (EWFD Directive 2008/105/EC).

**Nonylphenol and bisphenol A:** NP is mainly used for the synthesis of nonylphenol ethoxylates (NPEOs), which are applied as surfactants, e.g., in industrial cleaners, or as emulsifiers, e.g., in paints and lacquers, adhesives and pesticides. NPEOs are degraded to NP in the environment.
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NP is known to be slowly degradable and bioaccumulative with a far greater toxicity than NPEO (German Environmental Specimen Bank 2012). BPA is a synthetic monomer with one of the highest production yields worldwide, normally used in the production of polycarbonate plastics and epoxy resins (Diamanti-Kandarakis et al. 2009). NP and BPA are linked to a wide variety of endocrine dysfunctions. NP is known as an endocrine disruptor causing harmful effects including feminization and carcinogenesis in various organisms (Soares et al. 2008). Exposure to BPA can increase the risk of mammary cancer, obesity, diabetes, and reproductive and neuroendocrine disorders (Diamanti-Kandarakis et al. 2009).

**Water:** Shao et al. (2005) detected NPEOs at a range of $6.9 \times 10^3$ to $97.6 \times 10^3$ ng/L in April and $2.5 \times 10^3$ to $52.7 \times 10^3$ ng/L in December, as well as NP from $1.7$ to $7.3 \times 10^3$ ng/L in July in Yangtze River and Jialing River at Chongqing section. But corresponding drinking water samples derived from river water sources suggested that conventional water treatment processes had a significant removal efficiency of NPEOs ($> 99\%$) and NP (62-95\%). However, the drinking water still exhibited levels of NP in a range of $0.1 \times 10^3$ to $2.7 \times 10^3$ ng/L, to which attention needs to be paid (Shao et al. 2005). Shao et al. (2002) reported NP concentrations in Yangtze River also at Chongqing section ($20-6.9 \times 10^3$ ng/L), which were far greater than levels in the Yangtze Estuary and adjacent areas, ranging from 13 to 186 ng/L (Ping 2011). The concentrations of NP at Jialing River and Yangtze River at Chongqing section were above the regulatory limit (300 ng/L) set forth by European Union (EWFD Directive 2008/105/EC). Wang et al. (2012a) investigated NP and BPA concentrations in surface water from Yangtze River, Suzhou River and Huangpu River, as well as drinking water sourcing from surface water of Huangpu River and Yangtze River in Shanghai section. No phenols were detected in surface water from Yangtze River, while BPA was positive in all surface water samples from Suzhou River and Huangpu River. The concentrations of BPA in surface water of Huangpu River ranged from 184 to 782 ng/L. BPA was also detected in some drinking water and barreled water samples, with concentrations of 6 to 432 ng/L and 14 to 280 ng/L, respectively. The authors emphasized that more attention needs to be paid to the water quality surveillance in the city of Shanghai (Wang et al. 2012a). BPA is subject to review for possible identification as priority substance or priority hazardous substance by the European Union (EWFD Directive 2008/105/EC). However, there is still a lack of regulatory limits for BPA in drinking water and surface water.

**Sediment:** Bian et al. (2010) reported the distribution characteristics of NP and BPA in surface sediments of the Yangtze River Estuary and the adjacent East China Sea. The contents of NP
and BPA in surface sediments ranged from 2 to 36 ng/g and 1 to 13 ng/g, respectively. The contents of NP in the sediment core ranged up to 21 ng/g in layers from 1971 to 2001 with a pattern that reflected the traces of economic development history in China during this period. The deposition fluxes of NP varied from 1 to 18 ng/(cm$^2 \times a$). BPA was detected in sediment layers deposited from 1973 to 2001 with contents of up to 4 ng/g. The fluxes of BPA varied from 1 to 3 ng/(cm$^2 \times a$) exhibiting a similar pattern as NP.

1.2.6 Discussion

This chapter summarizes and describes the levels of PAHs, PCBs OCPs, PCDDs/DFs and emerging pollutants (PBDEs, PFCs PAEs, NP and BPA) in water and sediment of the Yangtze River. The concentrations of organic pollutants in the environment reflect the rapid industrialization and increased urbanization seen in the vicinity of the Yangtze River in China over the last two decades. Some areas such as Wuhan section and the South Branch of the Yangtze Estuary posed a potential ecotoxicological risk according to current guidelines and require a long-term monitoring. Specific strategies should be employed to restrict the pollutant discharge in those areas.

Compared to the 1990s, the PAH pollution in the Yangtze River has dramatically increased due to anthropogenic activities. The concentrations of PAHs in water of the Yangtze River were significantly high at Panzhihua section of the Jinsha River and comparably low in the TGR region. The concentration of BaP in water, which is one of the most toxic PAHs, peaked at the sections of Jiangsu and Wuhan, but was not detected at all at Jinsha River. The highest concentration of PAHs in the sediments of the Yangtze River was found in the tributaries of Wuhan section and the Yangtze Estuary near Shanghai. Based on the comparison of two guideline values (ERL and ERM), PAHs detected at Wuhan section may cause toxic effects to aquatic life. The serious PAH pollution at Panzhihua section in Jinsha River could mainly be attributed to the waste discharge of some local coking plants and many coal chemical industries in the area. PAHs in Upper and Middle Reaches of the Yangtze River seemed to originate from the combustion of coal and biomass. The combustion of petroleum and vehicle emissions mainly contributed to the PAH contamination of the Yangtze Estuary and near-by shore areas. Furthermore, sewage discharge from cities like Chongqing, Wuhan and Shanghai are also an important input source of PAHs into the surrounding environment. All these regions should be supervised to minimize the output of PAHs.
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PCBs are toxic chlorinated hydrocarbons, which mainly originated from the import of contaminated electronic devices and former national production. Except at the outlet of Nanjing city, the concentration of PCBs in water of the Yangtze River is below the quality standards enacted by China’s Ministry of Environmental Protection. High concentrations of PCBs were found in sediments at Wuhan section. Due to low solubility and high octanol-water partition coefficients, they can easily accumulate in sediments. The guideline value (ERL) suggests that most of the PCB levels in sediments of the Yangtze River at Wuhan section and the Yangtze Estuary may cause adverse biological effects, though they are below the “relevant contamination” recommended by ICPR (2009). Most PCBs in the sediments of the Middle and Lower Reaches seemed to originate from land runoff and contaminated e-wastes during the past two decades.

DDTs and HCHs are important constituents of OCPs, which cause severe health problems. DDTs and HCHs could be detected in Tuotuo River, which is the origin of the Yangtze River, but neither of these pollutants has been used in that area. This could be explained by “cold condensation” and “the grasshopper-effect” of OCPs by atmospheric transport and sedimentation. In all sections of the Yangtze River, the levels of DDTs and HCHs are below the quality standards for surface water enacted by the China’s Ministry of Environmental Protection and DDTs regulatory limits imposed by the Europe Union. However, the concentrations of DDTs in sediments of the Yangtze River at Nanjing section and the Yangtze Estuary are above the guideline value (ERL), indicating that this may have ecotoxicological impacts. The α-HCHs/γ-HCH ratio suggests that HCHs, prevalent at Wuhan section, derived mainly from the usage of technical HCH in the past decades. However, there may be other input sources of lindane (γ-HCH) in this area. Moreover, DDT residues mostly derived from the usage of technical DDT over the past decades in the middle and Lower Reaches of the Yangtze River. Furthermore, the usage of dicofol in some agricultural areas should be carefully regulated to reduce the input of DDTs into the surrounding environment. There were only limited studies on PCP contamination in the Yangtze River. A low level of PCP in sediment of the Yangtze River was reported. However, the risks from independent and joint effects of PCP and associated PCDDs/DFs impurities have to be considered.

Because of limited equipment and limited trained personnel, PCDDs/DFs have not been thoroughly investigated in the Yangtze River, unlike other pollutants such as PAHs and OCPs. PCDD/DF levels in water at TGR are relatively low. PCDD/DF levels in sediments of the Yangtze Estuary were lower than those in the Pearl River, but were marginally higher than the
concentrations in Yellow Estuary and Mondego Estuary (Spain). The most abundant congeners of PCDD in water and sediments were OCDD, indicating that the residues of PCDDs/DFs in the Yangtze River were derived from the usage of Na-PCP in 1960s. Due to the combustion of petroleum and coal, and the incineration of solid wastes in China (Zheng et al. 2008), a continuous monitoring of PCDD/DF concentrations in water and sediments in the Yangtze River are urgently needed.

Large amounts of imported e-waste have become the most important input sources of PBDEs in China. No studies reported on PBDEs in water of the Yangtze River. Lower concentrations of PBDEs in sediments were detected at the Lower Yangtze Reaches. This was attributed to dilution effects, due to huge amounts of water and suspended matter from the Upper Reaches (Chen et al. 2006). BDE-209 is the predominant PBDE in the Yangtze River. The PBDE contamination of coastal sediments of the East China Sea was partly attributed to the Yangtze River. Stronger contaminated sites were deposition areas for PBDE containing fine-grained sediments discharged by the river.

PFCs have been listed as POPs at the Stockholm Convention 2009. The production of PFCs in China has increased 4 times from 2003 to 2006 and reached to more than 200 tons. Two of eight PFC factories in China are located at the Yangtze River (Wuhan and Shanghai). High PFOA levels were reported at TGR (Xituo section and Fengdu section), indicating that input sources were located around these areas. The Yangtze River at Wuhan section and Huangpu River in Shanghai also showed high PFOA values, which stood in accordance with locations of PFC factories in both cities. The highest PFOS levels could be detected in water of the Yangtze Estuary’s South Branch, at a site that served as freight terminal and passenger wharf. However, at Chongming Island high levels of PFOS were detected in sediments. Pan and You (2010) reported a high correlation between salinity and the affinity of PFOS to sediment at the Yangtze Estuary. Thus, it is to be expected that PFOS from upstream sources will accumulate in sediments of the estuary due to the dramatic change in salinity.

Furthermore, also available information about PAEs, NP and BPA in the Yangtze River were reviewed, indicating a potential risk to some areas. The maximum concentrations of DBP and DEHP in water of the mainstream at Chongqing and Wuhan section, as well as the Yangchenghu Lake in the Yangtze Delta have exceeded the surface water quality standard of China. Endocrine disrupting chemicals like BPA were reported in some drinking water samples and barreled water samples in Shanghai. Thus, attention should be paid to the water quality surveillance.
Emerging pollutants are not necessary new chemicals, but also substances that may have been present in the environment already for a long time while their presence and significance has just been realized within the past two decades. Data about the presence of emerging pollutants in the Yangtze River are still scarce. There is a long way to monitor and regulate emerging pollutants in the Yangtze River, considering that the analytical methods are still at an early stage of development and regulatory criteria for some emerging pollutants in water/sediment are still under discussion. Though China has built the organic pollutants management framework in the past two decades, it still lags behind developed countries with regard to the control of certain POPs and emerging pollutants in water and sediments (Wang et al. 2005). Two guideline values (ERL and ERM) can provide a basis for estimating potential biological impacts associated with chemical analysis of pollutants in the sediment (Long et al. 1995). However, limitations exist between the incidence of adverse biological effects and the level of selected pollutants. Some pollutants can even induce responses although they are below the limit of quantification. In addition, chemical contaminants in sediments rarely affect organisms as single substances, but instead cause adverse effects as diverse mixtures (Ahlf et al. 2002). Integrated evaluation methods, such as the triad approach (Chapman 1990, Chapman & Hollert 2006), effect-directed analysis (Hecker & Hollert 2009) and the hierarchical assessment on contaminated sediment method (Ahlf et al. 2002) can be employed in the ecotoxicological assessment of Yangtze River.
1.3 Effect assessment of water and sediment (*in vitro/in vivo*)

The chemical analysis of pollutants is often not able to explain ecotoxicological effects of complex environmental samples. Risk assessment based on concentrations, e.g., of priority pollutants in sediments or water, cannot reflect the risk of the actual mixture of contaminants, but only the risk of those pre-selected toxicants. Thus *in vitro* and *in vivo* test systems with endpoints like genotoxicity, mutagenicity and endocrine disruption are taken into account. The application of bioassays indicating effects on cellular, organism or population level in laboratory test systems and the availability of concepts for linking measurable effects of complex environmental samples to distinct toxicants are required to bridge the gap between the chemical contamination and ecological status (Brack et al. 2007, Gerbersdorf et al. 2011).

1.3.1 Genotoxicity and mutagenicity

Several substances, like PAHs, PCBs and PFCs, are known to possess mutagenic or genotoxic properties, which influence the genome of an organism and thereby can lead to severe impacts on health, e.g., cancer formation (ATSDR 2000, 2009, Stahl et al. 2011). Mutagenicity describes permanent changes in the structure and/or amount of the genetic material of an organism that can lead to heritable changes in its function. It includes gene mutations as well as structural and numerical chromosome alterations (Eastmond et al. 2009). Genotoxicity refers to the capacity to give rise to mutations, but is not necessarily associated with it. It includes all directly or indirectly mediated adverse effects on genetic information, e.g., damage to DNA and/or cellular components regulating the fidelity of the genome, like spindle apparatus, topoisomerases, DNA repair systems and DNA polymerases. Genotoxic events are reversible due to cellular repair processes. Thus not all genotoxic events become evident as mutations (Eastmond et al. 2009, UKCOM 2011). Sediments, contaminated with mutagenic substances, pose a hazard to indigenous biota (Chen & White 2004). Yet only limited research investigating genotoxic hazards of aquatic sediments using standardized bioassays (e.g., Ames assay with *Salmonella typhimurium* (Reifferscheid et al. 2012), micronucleus assay (Reifferscheid et al. 2008) and Single Cell Gel Electrophoreses (SCGE/comet) assay has been employed in the river.

**Upper Reaches:** Shu et al. (2002) and Qiu et al. (2003) both investigated the mutagenicity of source water in Chongqing section of the Jialing and the Yangtze River, using the Ames and SCGE assay, respectively. In the study by Shu et al. (2002), the mutation rate of the *Salmonella typhimurium* strains TA98 (frameshift mutagen indicator) and TA100 (baseshift mutagen indicator) were used as parameters to assess surface water quality. They concluded that most
organic extracts from the two rivers possessed mutagenic potential. The source water of most sites exhibited the highest mutagenicity during spring and Jialing River extracts showed comparably higher mutagenicity than those from the Yangtze River. The latter observation matched the SCGE assay results by Qiu et al. (2003). The authors attributed the more serious pollution in Jialing River to agricultural and industrial discharges, domestic sewages and a poor self-purification capacity compared with the Yangtze River. It was also detected that the chlorinated drinking water possessed much higher mutagenic potential than the source water, indicating that chlorination during water treatment may have produced disinfection byproducts, which enhanced the DNA damage in the bioassay.

**Middle Reaches:** Yuan et al. (2005) tested genotoxic effects in human HepG2 cells in the comet assay with chlorinated drinking water extracts from the three main water bodies Dong Lake, Han River and the Yangtze River at Wuhan section. The results suggested that genotoxicants and/or genotoxic disinfection byproducts were present among the three water bodies. The DNA damage in Han River was more serious than in Dong Lake and the Yangtze River. Lu et al. (2004) also studied genotoxic effects of chlorinated drinking water processed from raw water from Dong Lake and Yangtze River at Wuhan section, applying the comet assay (HepG2 cells) and the micronucleus assay. Results revealed that drinking water produced from polluted raw water using chlorination caused a significant and dose-dependent increase of DNA damage in the human cell lines. Also, chlorinated water samples collected in the cold season (March) showed higher micronuclei frequencies than samples in the high summertime (August). However, the lowest concentration (lowest observed effect level) that caused a significant increase in DNA migration in the comet assay was ten times lower in Dong Lake, and a hundred times lower in Yangtze River for samples taken at the same location in August than in March.

Dong et al. (2010) compared the mutation rate of nonvolatile organic compounds (NVOCs) of source water and processed water extracts, from the Yangtze River and Han River in the Ames assay during normal (March), low (December) and high (July) flow period. The study revealed that the tap water was stronger mutagenic than the source water, and that the mutagenicity of Han River was higher than of the Yangtze River. During normal flow period all samples exhibited mutagenicity in strain TA98 with and without S9-mix (a rat liver homogenate as an exogenous metabolic activation system), except finished water sourcing from Yangtze River was negative to TA98 with S9. During low flow period only the finished water and the terminal tap water of Yangtze River were positive to TA98 without S9. In high flow period, all samples were positive to TA98 with or without S9, except the source water of Yangtze River. None of
the samples from the different periods was positive to TA100 with or without S9. The results showed that the NVOCs mainly contained frameshift mutagens.

**Lower Reaches:** Shen et al. (2003a) collected tap water and source water samples in different areas of Shanghai, sourcing from Yangtze River and Tai Lake. Testing the samples in the Ames, Arabinose resistance test (*Ara* test) and *SOS/umu* test proved that tap water samples (sampled in south and middle Shanghai) originating from Tai Lake possessed mutagenic potentials, whereas two tap water samples (sampled in north Shanghai) originating from Yangtze River, were not mutagenic. Shen et al. (2003a) also found that the water displayed an even stronger mutagenic potential, compared to its original tap water after boiling. Alike Shu et al. (2002), Dong et al. (2010) and Wu (2005), the authors referred the molecular mechanism of mutagenicity to be associated with a frame-shifting potential. Chlorinated tap water extracts from city middle and their corresponding source water were compared with respect to their chemical composition. Gas chromatography-mass spectrometry (GC-MS) analysis revealed qualitatively similar spectra, except for the peaks of three chlorinated aromatic hydrocarbon compounds (3,4-dichloroaniline, 2,4-dichloroaniline, 2,6-dichloro-4-nitroaniline), which existed only in the tap water. The authors concluded that the chlorination step lead to the creation of more toxic compounds, including mutagens. The authors stated that cancer risks may be elevated in areas where heavily chlorinated water is consumed. Li et al. (2006) investigated genotoxic effects in the comet assay with human peripheral blood lymphocytes and the micronucleus test with *Vicia faba* root tip cells after exposure to organic extracts from water of Yangtze River at Nanjing section. DNA damages in lymphocytes and root tip cells indicated mutagenic potentials at this section. Wu (2005) investigated the genotoxicity of surface water samples from Yangtze Estuary with the Ames assay. Genotoxicity was detected in several samples by the strain TA98, while no response could be detected in all samples with strain TA100. The mutation rate increased when S9-mix was added. This indicated that the mutagenic potential of the estuarine water samples was manifested in the bacteria’s genome by the mechanism of frame shifting (strain TA98). The water of the Yangtze Estuary’s South Branch and some samples from the seaward end of the estuary exhibited genotoxic potentials. The responsible toxicants were both direct (- S9 mix) and indirect (promutagenic) (+ S9 mix) mutagens (Wu 2005).
1.3.2 Endocrine-disrupting activity

The groups of endocrine disruptors are composed of a large variety of molecules. Besides natural (e.g., 17β-estradiol) and synthetical hormones (e.g., 17α-ethinylestradiol) as well as natural components (e.g. phytoestrogens like genistein), this group compound include synthetic chemicals used as industrial solvents/lubricants and their byproducts (PCBs, polybrominated biphenyls [PBBs] and dioxins), plasticizers (BPA, phthalates), pesticides (methoxychlor, chlorpyrifos, DDT), fungicides (vinclozolin), and pharmaceutical agents (diethylstilbestrol [DES]) (Diamanti-Kandarakis et al. 2009). Due to their influence on the hormone (endocrine) system, endocrine disruptors are known to have an impact on wildlife health (e.g., feminization of fish and masculinization of snails) (Sumpter & Jobling 1995, Jobling et al. 1996). Chronic exposure to low concentrations (5-6 ng/L) of 17α-ethinylestradiol even lead to the almost extinction of fathead minnow in one Canadian lake (Kidd et al. 2007). In addition, endocrine disruptors are also hypothesized to have effects on human health as well (e.g., male and female reproduction, breast development and cancer, prostate cancer, neuroendocrinology) (Diamanti-Kandarakis et al. 2009). Specifically, the USA, Japan, EU, and OECD have established testing approaches and regulatory frameworks with aim to assess the risks associated with chemicals that have endocrine disrupting properties (for review Hecker & Hollert 2011). There is evidence that endocrine disruptors have been detected in the Yangtze River. Nonylphenol (NP) was reported in river water, drinking water and fish tissues in Chongqing section (Shao et al. 2005) and natural estrogens estrone (E1) and 17β-estradiol (E2) were detected in municipal wastewater extracts at Nanjing section (Lu et al. 2010b). Furthermore, NP and BPA were also detectable in surface sediments of the Yangtze Estuary and the adjacent East China Sea (Bian et al. 2010). To estimate the ecotoxicological effects bioassay studies have been performed.

**Upper Reaches:** Zhu et al. (2003) assessed the estrogenic activity of organic extracts from water of Chongqing section with the cell proliferation test with MCF-7 cells. The samples were taken at five water treatment plants sourcing from the Jialing River (upstream and central city section) and the Yangtze River (upstream, central and downstream city section after confluence). Estrogenic activity was detected in all samples, with higher activity at the central and downstream sections than in the upstream sections of Chongqing. The authors attributed the effects to the discharge of sewage and industry wastewater from Chongqing. The estrogenic activity was higher in summer than in winter, which was attributed to stronger runoff from agricultural fields during the rainy season in this time of the year (Zhu et al. 2003). It might also be possible that estrogenic active compounds were remobilized from agricultural fields on the
riverbank or from waste that accumulated along the shore, both being dry during low and
undulated during high water period. Di-iso-butyl-phthalate (DiBP) and DBP were identified
as the main pollutants (Tian et al. 2003).

**Middle Reaches:** The NVOCs in samples of the source water and tap water from the Yangtze
River and Han River in Wuhan section were investigated by Dong et al. (2010) with the Yeast
Estrogen Screen (YES) assay in low, normal and high flow periods. Only the source water
samples of the Yangtze River were estogenic active in low flow period. During normal flow
period both the source water samples of the Yangtze River and the Han River showed estogenic
activity, with a higher activity in the Yangtze River samples. No water sample showed
estogenic activity in high flow period. Because activity could only be found in the source water
samples, the estogenic activity of NVOCs was most likely erased by the routine process of
water works.

**Lower Reaches:** The estogenic content of the Yangtze River in Nanjing section was assessed
in an *in vivo* bioassay with adult male goldfish (*Carassius auratus*) (Lu et al. 2010a). The assay
revealed significant serum vitellogenin (VTG) and E2 induction as well as gonad atrophy in the
treated fish. The result was consistent with the levels of water estrogens determined by chemical
analysis. Steroidal estrogens were the major causal agents responsible for the estogenic
responses in the Jiangxinzhou and Daqiao sections, while phenolic estrogens were the main
contributors in the Sanchahe section. Song et al. (2010) held polar contaminants responsible for
the estrogenic activities in Jiaxingzhou and Daqiao section, while mid-polar and nonpolar
contaminants led to the majority of estrogenic activities in Sanchahe section. The estogenic
activities in this area originated from the effluents of wastewater treatment plants.

In addition to the mentioned estrogenic activity, thyroid hormone (TH) agonist and antagonist
activities of water sources along the Yangtze River between Nanjing and Nantong (near
Shanghai) were examined by a green monkey kidney fibroblast (CV-1) cell-based TH reporter
gene assay (Shi et al. 2011). Responsible thyroid-active compounds were determined by
chemical analysis. To predict the TH agonist and antagonist activities instrumentally derived
L-3,5,30-triiodothyronine (T3) equivalents (T3-EQs) and thyroid receptor (TR) antagonist
activity equivalents referring to dibutyl phthalate (DBP-EQs) were calculated from the
concentrations of individual congeners. It could be shown that only one water source from
Nanjing section and two water sources from the section between Taizhou and Changzhou
contained TR agonist activity equivalents (TR-EQs), ranging from 286 to 293 ng T3/L. On the
other hand anti-thyroid hormone activities were found in all water sources with the TR

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antagonist activity equivalents referring to DBP (Ant-TR-EQs), ranging from $51.5 \times 10^6$ to $55.5 \times 10^7$ ng/L. The comparison of equivalents from instrumental and biological assays led to the conclusion that the TR antagonist activities might be attributed to high concentrations of DBP and di-2-ethylhexyl phthalate (DEHP) at some locations of the Yangtze River (Shi et al. 2011).

1.3.3 Other endpoints

This chapter summarizes the outcome of those studies, obtained from the available articles in the scope of this review, which investigated endpoints that did not belong to any of the former categories.

Upper Reaches: Organic extracts from surface water of the Yangtze River and Jialing River at Chongqing section, sampled during high (August) and low water period (January), have been examined by Cui et al. (2009) with respect to the endpoints ethoxyresorufin-O-deethylase (EROD) activity, cytochrome P-450 1A1 (CYP1A1) mRNA expression, aryl hydrocarbon receptor (AhR) binding capacity and activation of xenobiotic response element (XRE). The according methods were the EROD assay, reverse-transcription polymerase chain reaction (RT-PCR) and electrophoretic mobility shift assay (EMSA) applied to H4IIE rat hepatoma cells. The sampling sites were both located in urban areas near water supply sites of Chongqing city without any significant industrial discharges nearby. The main pollutants originate from traffic emissions and domestic sewage. The EROD assay revealed toxic equivalency (TEQ) values from $1 \times 10^{-4}$ to $13 \times 10^{-4}$ pg 2,3,7,8-TCDD/L river water. The low water samples exhibited higher TEQ values than the high water samples from the same site, and Jialing River samples showed higher TEQ values than samples from the Yangtze River from the same sampling season. However, overall the TEQ values were categorized to be relatively low. The time-dependent induction of CYP1A1 also demonstrated a difference between the seasons. An induction could still be measured in low water period samples after 48 hours, whereas the high water samples already showed no response at this time. PAHs were considered to be the responsible contaminants for the CYP1A1 induction, which were quantified by GC-MS analysis in a range from 231 to 994 ng/L. The Yangtze River samples from low water period contained the highest total PAH concentrations. The authors attributed the differences between the seasons to a lower flow rate in the low water period, which led to an enrichment of organic pollutants in the rivers.
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In a study by Li (2006), a subchronic toxicity test on rats was adopted to assess the risk of Chongqing drinking water samples - originating from the Jialing River - to humans. Pathological changes, hematological parameters, analysis of urine, biochemical serum parameters and an index related to free radical damages were observed at different doses. Genetic damage in body cells as well as reproductive cells could be shown. Although the organic extracts had potential toxicity on many organs the authors stated that with respect to the dosage of people’s daily intake the water might be safe for human usage (Li 2006). Another toxicity test on the reproductive system of male mice (*Mus musculus*) with organic extracts from tap water originating from the Jialing River suggested that mid and high doses of organic extracts could disturb the male reproductive system in mice. The number of epididymal sperm in the high organic extracts group as well as serum testosterone and follicle-stimulating hormone levels in the treated groups decreased significantly (p<0.05), whereas the frequency of sperm abnormalities in all treated groups increased significantly (p<0.05). Besides significant decrease of acid phosphatase and increase of γ-glutamyl transpeptidase activity (p<0.05), histological changes were observed in the mid- and high-dose organic extracts-treated groups (Zhao et al. 2011).

**Lower Reaches:** Another toxicological study with male mice (*M. musculus*), concerning the Yangtze River at Nanjing section as a source of drinking water, demonstrated that the source water had a toxic impact on the reproductive system of the tested animals. Alterations in different germ cell populations were observed as well as a significant increase of the percentage of abnormal sperms. Furthermore, there were obvious testicular histopathology distinguishes noticed in expansion of interstitial space and reduction in the number and size of leydig cells (Zhao et al. 2009).

Wang et al. (2010b) exposed goldfish (*C. auratus*) to organic water extracts taken near inlets of main branches or the outlets of wastewater treatment plants at three representative sections (Daqiao, Sanchahe and Jiangxinzhou) of the Yangtze River in Nanjing section. Acetylcholinesterase (ACHE), glutathione S-transferase (GST), EROD, glutathione peroxidase (GPx) and Na+/K+-ATPase activities were determined and alterations could be observed. EROD and GST activities appeared to be the more sensitive biomarkers. The levels of ACHE, GST, EROD, Na+/K+-ATPase activities changed with the change of the extracts polarity. Most significant responses were detected for fractions with intermediate and weakly polar components. To evaluate the impact the integrated biomarker response index (IBR) was calculated. The authors concluded that wild fish at Nanjing section of the Yangtze River were
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at potential ecological risk. Water extracts from Jiangxinzhou exhibited greatest adverse biological effects, followed by Sanchahe. Samples from Daqiao possessed the comparably lowest adverse potencies (Wang et al. 2010b).

Furthermore, organic extracts of surface water of the Tai Lake section in Jiangsu Province of the Yangtze River was investigated by Wang et al. (2011), with *in vitro* cytotoxicity assays (MTT cell viability test, lactate dehydrogenases release and annexin V–PI staining combined with flow cytometry) applying the sertoli, leydig and spermatogenic cells from adult male Sprague–Dawley rats. In addition, selected pollutants including PCBs, OCPs and PAHs were quantified by instrumental analysis. Though the total concentration of PAHs did not exceed national drinking water source quality standards (Ministry of Health - China 2006), the concentrated organic extract (286-fold) induced significant reproductive toxicity. PAHs were considered responsible to pose the greatest risk of the chemicals studied. The authors stated that the extract was enriched to determine if there were adverse effects due to exposure to interactions among measured residues or from unquantified residues that might have been in the mixture (Wang et al. 2011). As an example enrichment factors for OCPs were 39 to 59 fold and for PCBs were 510 to $42 \times 10^3$ fold in fish of the Yangtze River in Jiangsu area (Hu et al. 2009b). The results indicated that chronic exposure to the water can cause adverse effects on the male reproductive system, e.g., by bioconcentration of the compounds present in the water (Wang et al. 2011). Wu et al. (2010) evaluated the sediment quality of the Yangtze Estuary using zebra fish (*Danio rerio*) embryos. The chemical analysis revealed that the concentrations of zinc (Zn) and fluorene in the sediment samples were $2.4 \times 10^5$ ng/g and 46 ng/g, respectively. According to the Sediment Contact Assay and Fish Embryo Toxicity Test, the survival rate and heart rate of zebra fish embryos were reduced, while abnormalities and delayed hatching were induced, indicating that the sediment from the Yangtze Estuary may have teratogenic effects on biota.

1.3.4 Discussion

The assessment of environmental samples focused mainly on mutagenicity/genotoxicity (Ames, comet and micronucleus assay) and endocrine effects (Cell proliferation, YES and *in vivo* assay with *C. auratus*) in the compartment water, which has to be differentiated into source/surface water, tap water and drinking water. The main sections of interest were the same that were predominantly investigated with respect to pollutant levels in water and sediment: the highly populated areas of Chongqing in the Upper Reaches, Wuhan in the Middle Reaches, as well as Nanjing and the Yangtze Estuary in the Lower Reaches. A large amount of citizens,
who require also a large amount of unpolluted drinking water and food, as well as main polluters that discharge contaminants into the local rivers, like industry and domestic wastewater, are concentrated in these areas. Thus, human health is there at a particular risk. Based on the reviewed articles it can be stated that the inflowing Jialing River at Chongqing in the Upper Reaches, though both rivers possess mutagenic and endocrine potentials, seems to be more seriously polluted than the Yangtze River. This was mainly attributed to agricultural, industrial and domestic discharges along the Jialing River, as well as a poor self-purification capacity compared to the Yangtze River (Qiu et al. 2003). Water from the Yangtze River, Dong Freshwater Lake and the Yangtze feeding Han River at Wuhan section in the Middle Reaches showed mutagenic and endocrine activity as well. The tributary in this section possessed also a higher mutagenic potential than the mainstream (Dong et al. 2010). Alike the other sections the Lower Reaches revealed mutagenicity and endocrine activity in the Yangtze River water at Nanjing and the Yangtze Estuary. According to mutagenicity the South Branch of the estuary seems to be more seriously polluted than the North Branch (Wu 2005).

Another interesting observation was that all Ames mutagenic assays match in the outcome that especially frameshift mutagens were the responsible inducers (Shu et al. 2002, Shen et al. 2003b, Wu 2005, Dong et al. 2010). Similar observations were made in the German Rhine River (Kosmehl et al. 2004). In addition, genotoxicity was increased when the metabolically active S9-mix was added, indicating a predominant presence of promutagenic substances (Wu 2005). The pattern of genotoxicity was comparable to that previously described by Dobias et al. (1999), who applied the Ames assay in an effect-directed chemical analysis to measure the differences in mutagenicity of the main and subfractions of extractable organic material in the ambient air at workplaces of a coke oven. Based on these results they concluded (1) that most of the mutagenicity found in the main fractions required metabolic activation in vitro (+ S9 mix) and (2) frameshift mutations were the predominant type of mutation observed. The authors attributed the most important role to the carcinogenic PAHs and genotoxic nitrocompounds (Dobias et al. 1999). The frameshift mutagenicity of nitro-PAHs has also been proven in other studies (McCoy et al. 1981, Xu et al. 1982) and they were considered to be among the main responsible components for mutagenicity in Danube River sediments in Germany (Higley et al. 2012). Nitro-PAHs emerge from the emission of combustion sources and form in the environment from reactions of certain PAHs, e.g., phenanthrene, with the highly reactive nitrite (NO$_2^-$). The latter is produced mainly naturally through biological processes, like nitrification (ammonia oxidation) and denitrification (nitrate reduction). Oxygen deficiency or pollution with nitrogenous waste, e.g., from fertilizers, promotes the creation of nitrite. Nitrite has already
been proven to be toxic to freshwater fish, including carps and catfish (Lewis Jr & Morris 1986). In addition to that can it enhance the toxicity of PAHs by causing severe hepatic damage in fish and impact the metabolism of PAHs (Shailaja & Rodrigues 2003), as well as increasing the formation of mutagenic metabolites in fish, e.g., exposed to refinery effluent (Shailaja et al. 2006a,b). As previously described is the water and sediment in many sections of the Yangtze River polluted by PAHs, which primarily originated from combustion sources. This supports the assumption that the mutagenic activity in the Yangtze River region might be attributed to a high degree to PAHs and nitro-PAHs from combustion sources, as well as nitro-PAHs that form from polycyclic hydrocarbons in the aquatic environment. Yet further effect-directed analysis should be utilized to clarify what the responsible components are. Although mutagenic/genotoxic chemicals like heavy metals, PAHs, heterocyclic amines and pesticides were detected in surface waters all around the world in numerous studies, linking those to the measured mutagenicity has been difficult. Thus many major putative mutagenic/genotoxic compounds in most surface waters with high mutagenic/genotoxic activity in the world remain yet unknown (Ohe et al. 2004).

Furthermore, it was observed that chlorination of polluted source water during drinking water treatment increased its mutagenic potential (Shu et al. 2002, Shen et al. 2003b, Wu 2005, Dong et al. 2010), whereas the estrogenicity could be erased after the routine process of water works (Dong et al. 2010). Boorman (1999) pointed out that several mutagenic byproducts can originate from disinfection of drinking water, e.g., after chlorination. Chlorine gas (Cl₂) and hypochlorite are commonly used for chlorination processes in water treatment. Hypochlorite and hypochlorous acid (HOCI) are the main chlorine species under typical water treatment conditions (pH 6-9), with HOCI being the major reactive form. Hypochlorous acid originates from the hydrolyzation of Cl₂ in water. The main reactivity of chlorine results from electrophilic attacks of HOCl on organic and inorganic compounds (Deborde & Von Gunten 2008). The main disinfection byproducts resulting from the reaction of chlorine in drinking water with naturally occurring organic matter in water are trihalomethanes (THM) and haloacetic acids (HAA). Further attention is drawn to the family of chlorinated furanones, because most of the mutagenicity found in chlorinated drinking water can be referred to 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, a member of this family (WHO 1997, Boorman 1999). Variables that influence the formation of chlorine species and disinfection byproducts are chloride concentration, concentration of dissolved organic matter, pH, temperature and bromide concentration in the water (Deborde & Von Gunten 2008, Platikanov et al. 2010).
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Chen et al. (2010) measured THM and HAA concentrations in raw water and processed water of Yangtze and Huangpu River in Shanghai section. In addition to that they examined fluctuations during transmission and distribution in the pipeline network. While THM concentrations of finished water from Yangtze River (21-31 μg/L) exceeded those from the Huangpu River (7-9 μg/L), a reverse picture could be shown for HAAs (Yangtze: 19-23 μg/L; Huangpu: 25-37 μg/L). For matters of comparison, neither exceeded the maximum allowable annual average level given by the USEPA (THM: 80 μg/L; HAA 60 μg/L). The fluctuations of THM and HAA levels in the distribution network were low during transmission and distribution process.

The cancer risk originating from the formation of trihalomethanes in drinking water sourcing from the Yangtze River in Shanghai section was shown by Liu et al. (2011b). Based on an adopted human health risk assessment developed by the Environmental Protection Agency - USA (2005) it could be found that the total cancer risk (oral, dermal and inhalation) was highest in spring (male: $8.23 \times 10^{-5}$ meaning 82.3 cases/million persons; female: $8.86 \times 10^{-5}$) and lowest in summer (male: $3.57 \times 10^{-5}$; female: $3.84 \times 10^{-5}$). Levels for autumn (male: $5.38 \times 10^{-5}$; female: $5.79 \times 10^{-5}$) and winter (male: $5.69 \times 10^{-5}$; female: $6.15 \times 10^{-5}$) lay in between. The lifetime cancer risk of THMs was the highest via oral ingestion. The average concentration of THMs was lowest in autumn (66 μg/L) followed by winter (78 μg/L), summer (98 μg/L) and the highest level in spring (101 μg/L). Because these values demonstrate only one variable of the cancer risk assessment, beside chronic daily intake, exposure frequency, duration and others, the high difference in cancer risk can be explained between spring and summer despite comparable THM concentrations. The high level in spring was related to a higher bromide ion concentration resulting from the intrusion of tidal saltwater into the Yangtze River during the dry season. In addition to that it was found that the presence of Fe(III) increased the levels of THMs and subsequently the cancer risk to humans (Liu et al. 2011b). According to the guidance of Environmental Protection Agency - USA (1989), a cancer risk below $10^{-6}$ (1 case per million persons) is negligible and above $10^{-4}$ (100 cases per million persons) is considered to be sufficiently large that some sort of remediation is desirable. A cancer risk between $10^{-6}$ to $10^{-4}$ is classified as an acceptable risk (Environmental Protection Agency - USA 2013). The background cancer risk over a lifetime is 1 case in 3 persons (Environmental Protection Agency - USA 1997). Based upon this classification the cancer risk originating from THMs in the investigated drinking water is acceptable throughout all seasons (Liu et al. 2011b). Yet it has to be considered that the treated water bears a complex mixture of disinfection byproducts which add to the individual cancer risk of THMs. This includes, besides non-mutagenic dissolved
organic matter as parent substances, also the chlorination of pollutants like PAHs (Deborde & Von Gunten 2008). The primary generation of chlorinated PAHs (CIPAHs) is during pyrolysis and a secondary reaction process also occurs in the aquatic environment. Certain CIPAHs induce even higher mutagenicity and aryl hydrocarbon receptor activity compared to their corresponding parent PAHs (Ohura 2007). Colmsjö et al. (1984) could show that mutagenicity of the weak mutagenic PAHs pyrene could be multiplied after chlorination. Some CIPAHs are reported to induce tumorigenicity and oncogene activation (Fu et al. 1999). Shiraishi et al. (1985) demonstrated the presence of CIPAHs in concentrations of 10^{-1} to 10^{-2} ng/L in chlorinated tap water of Tsukuba, Japan. Shen et al. (2003a) discovered the chlorinated monoaromatic hydrocarbons 3,4-dichloroaniline, 2,4-dichloroaniline and 2,6-dichloro-4-nitroaniline in processed tap water, which were not detected in the corresponding source water, indicating a formation of these substances associated with the disinfection step. Beyond was observed that boiling of the mutagenic tap water led to even stronger mutagenicity. The authors referred this phenomenon to an accelerated chlorination of non-volatile organic residues by excessive chlorine in the tap water during the heating process. This is especially critical as it is widely believed, as in Shanghai, that boiling reduces the risk originating from the water (Shen et al. 2003a). Furthermore several epidemiological studies have suggested that drinking chlorinated water may be associated with increased incidences of bladder, rectal, and colon cancer (King & Marrett 1996, Koivusalo et al. 1997, Hildesheim et al. 1998) and adverse reproductive effects (Waller et al. 1998). To summarize, it should be considered that chlorination of non-mutagenic dissolved organic matter and other parent substances during water treatment adds to the total mutagenicity of components in the polluted source water.

Another striking observation was that the greatest mutagenic/genotoxic activities were observed during spring (March) in the majority of all cases. The Yangtze River passes annual fluctuations in its water level, which is influenced by the rainfall throughout the year. There is no clear definition about the timeframe for each flow period. As a rough classification, the water level is lowest in the dry season in winter/spring (December to April), at a medium level in spring/summer (May to June) as well as autumn (October to November), and highest in the rainy season in summer (July to September) with occasionally occurring flood events (Sun et al. 2002). The seasonality of the water level in the Yangtze River in the TGR section is inverted due to the construction of the TGD (Cui et al. 2009). Since the impoundment, the water level is lowest in summer (June to September) and rapidly rising towards the highest level over winter (October to March) with middle levels in spring (April to May). The inverted water levels are attributed to the buffer capacity of the reservoir to prevent flood events and supply the Middle
and Lower Reaches with water during the dry season. The observation that the highest mutagenicity/genotoxicity could often be observed in spring is most likely associated with higher pollutant concentrations in the water - due to lower dilution - during the low water period in this season. This assumption is supported by the observation that PAHs levels in the South Branch of the Yangtze Estuary during dry season in spring were comparably higher than those in the rainy season in summer, as described before (Ou et al. 2009). Also CYP1A1 inductions of surface water extracts from Yangtze River and Jialing River at Chongqing section were greater in low water period (January) than in higher water period (August). The authors attributed the differences between the seasons to a lower flow rate in the low water period, which led to an enrichment of organic pollutants in the rivers (Cui et al. 2009). Due to the inverted water level situation in the TGR since impoundment, it is to be expected that the changes of hydrology manifest as changes in contamination conditions, with higher pollutant and effect levels in summer during rainy season than during dry season (Cui et al. 2009), caused by a lesser dilution and reduced velocity in the reservoir. Hydrophilic compounds may be carried with the discharged water past the dam, but hydrophobic substances will most likely remain in the sediments of the reservoir. It has also to be considered that the elevated precipitation in summer is associated with an increased amount of air-borne particles washed out from the air as well as a stronger runoff from fields, carrying pollutants into the TGR, which may also be trapped there and subsequently accumulate in this region.

As PAHs seem to play a major role in the Yangtze River Region it is surprising that only limited research was performed on biomarkers or bioassays displaying the cytochrome P450 activity, a major enzyme family for the metabolism of lipophilic xenobiotics. PAHs, PCBs, dioxins and furans are metabolized by the CYP1A enzyme subfamily. Their activity can be measured, e.g., by the EROD assay, which is widely applied and accepted to test dioxin-like activity of water and sediment in vitro as well as to measure the biochemical response in organisms in vivo and in situ exposed to the previously mentioned xenobiotics (Hilscherova et al. 2000, Whyte et al. 2000, Brack et al. 2005). Also more specific in vitro assays, like the H4IIE assay, are widely accepted and able to measure either enzyme activity (i.e., EROD) or agonistic receptor binding (e.g., PCBs, PAHs, PCDDs/DFs) at the AhR, which is considered to be involved in the mediation of dioxin-like effects (Poland & Knutson 1982, Tillitt et al. 1991, Villeneuve et al. 2001).

As most of the studies applied organic extracts in varying concentrations to the bioassays, there is a certain degree of uncertainty regarding the actual risks associated with the native samples.
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Therefore it is crucial to also conduct *in situ* studies in the same area, to bridge the gap between laboratory and the field (Chapman 1990). Regardless, analyses of organic extracts provide useful information with respect to the presence of compounds that may lead to effects after chronic exposure and/or bioaccumulation.
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1.4 Pollutant levels and adverse effects in aquatic organisms (in situ studies)

The fish fauna of the Yangtze River basin is one of the richest world-wide, offering plentiful fish resources (Fu et al. 2003, Chen et al. 2009), but at present these fishery resources are seriously depleted and the fishery yield is significantly reduced (Chen et al. 2009). The fishery yield in the Yangtze River grew from 1949 to 1954 and peaked at about 427,000 tons in 1954. The annual yields during 1955 to 1971 were stable at about 261,000 t per year and began to decrease to 200,000 t from the 1980s. In 1990s, the fishery yield was even halved to about 100,000 t (Chen et al. 2002a) (More recent numbers were not available). The fish community has been altered, with a reduction in the quantity of rare, peculiar and economically important fish species populations, an increase in the number of exotic fish species, decrease in migratory fish species and a severe trend in fish stunting. Habitat fragmentation and shrinkage, resources overexploitation, invasion of exotic species and water pollution were held responsible for these changes (Chen et al. 2009). Industrial wastewater and communal sewage discharge carrying toxicants are considered to induce the destruction of spawning grounds, depletion of brood stocks, decrease in production and even high mortality in fish (Chen et al. 2004). Moreover, the pollutants dislocate from the water column to particulate matter, and finally accumulate in sediments, which may play a role as secondary contamination sources (Brinkmann et al. 2010). The contaminants can accumulate in sediment-dwelling organisms, and be transferred to higher trophic levels through the food chain (Liu et al. 2003, Hu et al. 2009b). Bioaccumulation of toxicants may pose a potential health risk for local residents with regard to the consumption of contaminated food from the Yangtze River.

1.4.1 Pollutant levels in aquatic organisms

Upper Reaches: A major study carried out by Greenpeace (2010) evaluated the presence of PFCs (including PFOS) and alkylphenols (including NP and 4-tert-octylphenol [OP]) within commonly eaten wild fish (Cyprinus carpio carpio, Sillurus soldatovi meridionalis) from the Upper (Chongqing), Middle (Wuhan) and Lower (Ma’anshan and Nanjing) Reaches. These fish contained detectable levels of alkylphenols and PFCs. NPs were the predominant alkylphenols (present in over 95 % of carps, and over 85 % of catfish) and PFOS as dominating PFC. Whereas average NP levels were comparable in carp (23 ng/g ww) and catfish livers (24 ng/g ww) from Chongqing, OP (1 ng/g ww; 3 ng/g ww) and PFOS (< 0.3 ng/g ww; 22 ng/g ww) levels differed considerably. The carp samples from Chongqing were the only samples that did not contain any detectable PFCs. On the other hand catfish samples from the same area showed the highest OP concentrations (3 ng/g ww) (Greenpeace 2010). Shao et al.
(2005) also detected the bioaccumulation of NP and nonylphenol ethoxylates (NPOEs) in fish samples (Coreius guichenoti, Coreius heterodon, Leptobotia elongate, Rhinogobio typu, Rhinogobio ventralis) from Jialing River and Yangtze River at Chongqing section. The residual NP concentrations in muscle, gills, liver and stomach ranged from n.d. to 90 ng/g ww, 100 to 400 ng/g ww, 800 to $1.9 \times 10^3$ ng/g ww and 200 ng/g ww, respectively. While NPEO levels ranged from 400 to $1.3 \times 10^3$ ng/g ww for muscle, $2.1 \times 10^3$ to $5.8 \times 10^3$ ng/g ww for gills, $20.2 \times 10^3$ to $48.3 \times 10^3$ ng/g ww for liver and $2.2 \times 10^3$ ng/g ww in stomach, respectively. The authors compared these concentrations to levels of NP and NPEO in the surrounding water, gaining bioconcentration factors which indicated that NPEOs were more easily bioconcentrated in the fish than NP. Both chemicals concentrated especially in the fish livers. The authors concluded that under the assumption that one person takes in 200 g river fish tissues and 2 L drinking water, the maximum amount of NP ($390 \times 10^3$ ng/person/day or $65 \times 10^3$ ng/kg bodyweight) was far below the concentration that can elicit subchronic toxicity on laboratory rats (Cuny et al. 1997). Whereas the risk of NP was low from the view of human consumption they pointed out that measured NP levels in the Yangtze River were close to the threshold concentration ($10 \times 10^3$ ng/L) that affects fish reproduction (Jobling et al. 1996), thus posing a potential ecological risk.

**Middle Reaches:** Carp and catfish liver samples from Wuhan contained even higher average levels of the dominant alkylphenols and PFCs (carp: 85 ng NP/g ww; 2 ng OP/g ww; 42 ng PFOS/g ww; catfish: 32 ng NP/g ww; 40 ng PFOS/g ww), except for OP in catfish (3 ng/g ww). In comparison to the other sampling sites in this study highest NP (85 ng/g ww) concentration could be detected in carp from Wuhan (Greenpeace 2010).

**Lower Reaches:** Greenpeace (2010) could only investigate carp in Ma’anshan due to a scarcity of catfish during the sampling period. The carp samples contained lowest average levels of NP (6 ng/g ww) and OP (0.3 ng/g ww), except for PFOS (2 ng/g ww) which was higher than in Chongqing. The carp samples from Nanjing contained second highest average NP (34 ng/g ww), OP (2 ng/g ww) and PFOS (28 ng/g ww) concentrations compared to the carps from other study areas. Whereas the catfish showed the highest NP concentration (61 ng/g ww), they contained the lowest OP (2 ng/g ww) and PFOS (18 ng/g ww) average concentration among their species from the other sites (Greenpeace 2010).

Furthermore, PBDEs were determined in some species of aquatic biota from the Lower Reaches of the Yangtze River, including fish, crabs and shrimps (Xian et al. 2008, Gao et al. 2009, Su et al. 2010). The concentrations of PBDEs and hexabromocyclododecanes (HBCDs) in muscle
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of nine freshwater fish species (Hypophthalmichthys molitrix, Ctenopharyngodon idella, Aristichthys nobilis, C. auratus, Cyprinus carpio, C. heterodon, Parabramis pekinensis, Siniperca chuatsi, Channa argus) from the Yangtze River (near Nantong/Shanghai), reached to $1.1 \times 10^3$ ng/g and 330 ng/g (lipid weight), respectively (Xian et al. 2008). Findings of another study revealed that PBDEs and methoxylated PBDEs (MeO-PBDEs) were accumulated in four species of anchovy (Coilia nasus, Coilia mystus, Coilia nasus taihuensis, Coilia brachygnathus) from the Yangtze River (Nanjing to Yangtze Estuary), Tai Lake and Hongze Lake with concentrations up to 4 ng/g ww and up to 8 ng/g ww, respectively. The authors concluded that PBDEs in anchovy are primarily of synthetic origin and released by human activities, while MeO-PBDEs in anchovy primarily sourced as natural products from the sea, instead from metabolism of synthetic PBDEs in the animals itself (Su et al. 2010). Su et al. (2012) measured the concentrations of PBDEs and analogues in freshwater fish (C. carpio, C. auratus, Pelteobagrus fulvidraco, Hemiculter leuciscus, Coilia macrognathos Bleeker, Silurus spp.) from the Lower Yangtze Reaches and marine fish (Sinonovacula constricta, Drepane punctata, Ilisha elongate, Suggrundus meerdervoortii, Pseudosciacaena polyactis) from the Yellow Sea. Finally, the potential risk of these PBDE analogues was assessed by measuring the dioxin-like activity in the H4IIE-luc rat hepatoma transactivation bioassay. Most of the PBDE analogues were detected in the marine organisms. Of the eleven detected PBDE analogues, six were found to have measurable dioxin-like potency. Some PBDE analogues exhibited significant dioxin-like potency. However, concentrations of 2,3,7,8-TCDD equivalents, indicating the strength of potency, were less than the tolerance limit proposed by the European Union (2006) and the oral reference dose (RfD) derived by Environmental Protection Agency - USA (2012), respectively (Hazard Quotients [HQ] < 0.005). Although the PBDE levels in aquatic biota in the Lower Reaches of the Yangtze River and adjacent Yellow Sea did not exceed the safety levels, there should still be paid attention, because some analogues have been detected in other environmental media, as well as human blood (Su et al. 2012).

PFCs were measured in water and wildlife (C. auratus, Ictalurus punctatus, Pseudorasbora parva) and farmed fish (C. auratus, I. punctatus) as well as home-fed chicken and ducks collected from Shenyang (northeast China) and the Yangtze River Delta (Lu et al. 2011). The study aimed at the evaluation of the human health risk growing from the intake of PFOS and PFOAs via fish and domestic poultry dietary. The water of the Yangtze River Delta (PFCs: 42 to 170 ng/L; average river waters 105 ng/L; average surface seawaters 77 ng/L), with highest concentrations in the Shanghai section of the Yangtze River, exceeded the concentrations in water samples from the rivers in Shenyang (PFCs: average 5 ng/L). PFOA were the dominating
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compounds in both sections, in addition to PFOS in Shenyang. PFOS seemed to play only a minor role in the Yangtze River Delta. Urban sewage was claimed to be the main source of PFOS, and perfluorohexane sulfonate (PFHxS) in the investigated surface waters of Shenyang. In all biota samples PFOS (averages: Shenyang poultry 1 ng/mL serum and 1 ng/g dw liver; Shenyang fish 3 ng/g dw tissue; Chongming Island fish 6 ng/g dw tissue) and perfluoroundecanoic acids (PFUnDA) in fish (averages: Shenyang fish 1 ng/g dw tissue; Chongming Island fish 4 ng/g dw tissue) were the most abundant. The authors concluded that the acceptable daily intake of PFOS and PFOA through the diet of fish and poultry in the studied areas did not exceed the RfD for non-cancer health effects (hazard ratio values [PFOS; PFOA] < 1.0).

Wen et al. (2008) reported PCDDs/DFs in bivalves (*Limnoperna lacustris, Cobicula fluminea*) in the Yangtze Estuary. The concentrations of total PCDDs/DFs and the TEQs in bivalves varied between 0.3 and 1 ng/g dw as well as $3 \times 10^{-3}$ and $11 \times 10^{-3}$ ng/g dw, respectively. The biota-sediment accumulation factor (BSAF) values were studied, examining the relationship between concentrations of PCDDs/DFs in animal and sediment in this area. While it was found that the BSAF had an inverse correlation with the number of chlorines in PCDDs/DFs. According to the authors the herbicide PCP and Na-PCP and the waste discharge from a local sinter plant were held responsible to be the main source of PCDDs/DFs in the area.

A holistic approach to determine the accumulation of PCBs, PAHs, HCHs and DDTs in fish and benthos was performed by Hu et al. (2009b) at Jiangsu section. Fish (*C. carpio, H. molitrix, Ctenopharyngodon idellus*) and benthos (*Bithynia fuchsiana, Bellamya aeruginosa*) as well as water and sediment samples were taken between Jiangling (upstream Nanjing) and Haimen (Yangtze Estuary). The highest levels for PCBs, PAHs, HCHs and DDTs in fish were 23 ng/g, $7 \times 10^{-3}$ ng/g, $28 \times 10^{-3}$ ng/g and $76 \times 10^{-3}$ ng/g, respectively. Analogous to that have been the highest levels in benthos bodies 14 ng/g, $21 \times 10^{-3}$ ng/g, $26 \times 10^{-3}$ ng/g and $82 \times 10^{-3}$ ng/g. Four- to five- ring chlorinated biphenyls were chiefly detected among all PCBs, with PCB 105 being the major representative. According to the authors, the PAH concentrations have been higher in benthos ($21 \times 10^{-3}$ ng/g) than in fish ($7 \times 10^{-3}$ ng/g) including a significant positive correlation ($p < 0.05$), with two- to four- ring PAHs as the main components upon all PAHs measured. DDTs consisted entirely of $p,p'$-DDE isomers. Comparing the PCB burden in the fish to the environmental compartments sampled at the same sites, it was observed that fish from Jiangning, Jiangyin (halfway Nanjing and Shanghai) and Haimen accumulated PCBs with enrichment factors from $5.1 \times 10^{2}$ to $4.2 \times 10^{4}$ fold. Enrichment factors for OCPs ranged from
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3.9 \times 10^1 to 5.9 \times 10^1 fold in fish and 6.8 \times 10^1 to 1.46 \times 10^2 fold in benthos at the same sites. The authors concluded that this demonstrated the remaining presence and accumulation of already banished hazardous compounds in biota (Hu et al. 2009b).

The organochlorine pesticides HCHs and DDTs were detected in sediment-dwelling animals including mollusks (Corbicula fluminea, Sinonovacula constricta) and crabs (Sesarma dehaani) from the Yangtze Estuary and nearby coastal areas. Levels of HCHs ranged from 1 ng/g to 6 ng/g and averaged 4 ng/g in mollusks, while DDTs concentrations ranged from 26 to 69 ng/g, with a mean of 35 ng/g. In crabs HCHs concentrations varied from 2 to 26 ng/g and averaged 14 ng/g, whereas the concentrations of DDTs were in the range of 2 to 25 ng/g with a mean value of 6 ng/g (Yang et al. 2006). The concentrations of DDTs were far higher than those in sediments in this area (up to 1 ng/g, dw) (Liu et al. 2003). Liu et al. (2004) measured concentrations of OCPs in addition to PCBs in the same mollusks (C. fluminea, S. constricta, Bullacta exarata, Potamocorbula ustulata) and crab species (S. dehaani), as well as shrimp (Exopalaemon carinicauda) and fish (Mugil cephalus) also in the Yangtze estuarine and coastal areas. The gas chromatography-electron capture detector (GC-ECD) analysis determined HCHs concentrations ranging from 1 to 77 ng/g with a mean of 13 ng/g and with DDTs concentrations from 2 to 159 ng/g with a mean of 34 ng/g. The concentration distribution of PCBs ranged from 44 to 1.3 \times 10^3 ng/g with a mean value of 343 ng/g. Animals of the same species were characterized by higher contamination levels for males than females, and small individuals than large ones. The authors evaluated the contamination status of the animals to be at a moderate level. Another study by Ma et al. (2008) measured HCHs and DDT concentrations in shellfish (Oyster, Mussel, Mactra veneriformis Reeva, Meretrix meretrix Linnaeus, Scapharca suberenata) from the Yangtze Estuary and adjacent waters. GC analysis determined a concentration range of n.d. to 12 ng/g ww for HCHs (mainly alpha- and delta-HCH) and averaged at 1 ng/g, conforming to the first level of criterion (20 ng/g) of the Marine Biology Quality Criterion by the State Oceanic Administration - China (2001). For DDTs (mainly o,p'- and p,p'-DDT) the concentrations ranged from 4 to 282 ng/g with a mean of 58 ng/g exceeding the first level (10 ng/g), but conforming the second level (100 ng/g) of the Marine Biology Quality Criterion (State Oceanic Administration - China 2001). The highest concentration could be found at Shengsi (Island southeast of Shanghai), followed by Yangkougang (coast north of estuary - CNE), Lysi (CNE), Dongyuan (CNE) and Beibayao (Chongming Island). Low concentrations were observed at Changsha (CNE), Beidaodi (Southeast of Chongming Island) and Gouqi (Island southeast of Shanghai). Comparing the samples from 2006 and 2007 the concentration of HCHs and DDTs in most sites decreased, except for Yangkougang, Dongyuan,
Beidaodi, Lvsi, and Shengsi. Of all studied animals oyster had the highest bioaccumulation ability. Overall, it could be stated that the studied areas were slightly affected by OCPs, particularly by DDTs.

Chen et al. (2002c) measured the accumulation of petroleum hydrocarbons and volatile phenols in two migratory fish species (*Coilia ectenes, Eriocheir sinensis, Anguilla japonica*), three semi-migratory fish species (*Leiocassis longirostris, Pelteobagrus vachellii*) and five sedentary fish species (*Sillurus asotus, S. chuatsi, C. carpio, Megalobrama amblycephala, C. heterodon*) from the river sections of Anqing (halfway Wuhan and Nanjing) (*C. carpio, P. vachellii, M. amblycephala, C. heterodon*), Nanjing (*C. ectenes, E. sinensis, A. japonica, L. longirostris, S. asotus, S. chuatsi*), Zhenjiang (downstream Nanjing) (*C. ectenes, E. sinensis, A. japonica, L. longirostris, C. carpio, P. vachellii*) and the Yangtze Estuary (*E. sinensis*). Mean levels of petroleum hydrocarbons ranged in Anqing from $1.10 \times 10^3$ to $6.83 \times 10^3$ ng/g wet weight (ww), in Nanjing from $3.21 \times 10^3$ to $8.72 \times 10^3$ ng/g ww, in Zhenjiang from $3.25 \times 10^3$ to $9.23 \times 10^3$ ng/g ww, and in the Yangtze Estuary $2.83 \times 10^3$ ng/g ww could be detected. Mean levels of volatile phenols ranged in Anqing from 240 to 620 ng/g ww, in Nanjing from 520 to $3.18 \times 10^3$ ng/g ww, in Zhenjiang from 390 to $3.27 \times 10^3$ ng/g ww, and in the Yangtze Estuary 250 ng/g ww could be measured. The accumulation of these substances indicated that the area has been polluted by industrial effluents.

### 1.4.2 Adverse effects in aquatic organisms

**Lower Reaches:** Li and Shen (2010) measured the peripheral blood erythrocyte micronucleus rate of the wild *C. auratus* caught in the Yangtze River in Jiangsu Province (upstream Shanghai) as an *in situ* biomarker for genotoxicity. It was found that the genotoxic effects caused by the water of the cities Changshu (0.212 to 0.219 %; p < 0.01) and Jiangyin (0.199 to 0.200 %; p < 0.01) showed significant effects, while those of Haimen (0.144 to 0.152 %; p < 0.05) were comparably lower, yet still significant. Those from Jiayi (0.120 to 0.133 %; p > 0.05), Jiayin (0.120 to 0.133 %; p > 0.05), Jingjia (0.112 to 0.119 %; p > 0.05) and Zhengjiang (0.120 to 0.126 %; p > 0.05) were the lowest and revealed no significance compared to the control group (0.093 %). Chen et al. (2002c) also tested the formation of micronuclei in *C. carpio* from the river sections at Anqing (3.56 %), Nanjing (4.25 %), Zhenjiang (4.50 %) and the Yangtze Estuary (3.65 %). A high frequency of micronuclei (control group 0.82 %) indicated that the Yangtze River’s Lower Reaches possess high potential for adverse effects in the area. Micronucleus rates were also measured for *C. idellus* (3.55 %) and *L. longirostris* (4.68 %) from Nanjing and Zhenjiang, respectively, but no control
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was applied for those species. Chen et al. (2002c) pointed out that the observed formation of micronuclei might be associated with accumulated volatile phenol and petroleum hydrocarbons in the fish.

1.4.3 Discussion

Diverging from the research on the pollutant levels and effects in vitro and in vivo of water and sediment, the assessment of pollutant levels and adverse effects in wildlife organisms primarily focused on the Lower Reaches of the Yangtze River: from Nanjing to the estuary and the adjacent coastal area. The Upper and Middle Reaches were hardly investigated in this context. Upon the studies on biota those quantifying organic pollutants in the organism were higher in number than those investigating effects. Taking into account that cities like Chongqing and Wuhan have a major impact on the surrounding area, and as the bioassay studies already indicated ecotoxicological impacts in this area, further in situ studies are highly recommended. The reviewed articles chiefly focused on PFCs (mainly PFOS) and PBDEs in fish (mainly carp and catfish), as well as OCPs (mainly HCHs and DDTs) in mollusks and crabs. Yet there is only little knowledge available about the predominant priority pollutants that could be measured along the Yangtze River - like PCBs and PAHs – present in and their influence on aquatic organisms in this area. They have been demonstrated to potentially induce toxicopathic hepatic lesions in fish after long-term low exposure or short-term high exposure (Myers et al. 1998, Ortiz-Delgado et al. 2007).

With respect to toxicological endpoints the only response measured were genotoxic effects manifested in the formation of micronuclei in carp and catfish from the Lower Reaches. A broader spectrum of endpoints - like histopathology, immunotoxicology, biochemical alterations, and endocrine disruption - should also be taken into consideration along the whole river, especially in areas with elevated agricultural, industrial and domestic discharge levels. These information will help to further elucidate the reasons for the depletion of fishery resources and can be utilized to initiate counteractive measures (cf. Boettcher et al. 2010, Grund et al. 2010).

As wild fish and other aquatic organisms still remain an important dietary source in China (Su et al. 2012), bioaccumulation of pollutants in the aquatic organisms has to be considered. The accumulation poses a risk to the organisms on the one hand and on further consumers on the other, as shown by Scholz-Starke et al. (2013). The sustenance with polluted food, e.g., fish, muscles and crabs, might lead to secondary intoxication. This can either result in acute toxicity
or to effects like higher risk for cancerogenicity after chronic exposure to pollutant levels that exceed the acceptable daily intake (ADI). However, the reviewed articles revealed only a low risk of secondary intoxication with organic pollutants by consuming aquatic organisms from the Yangtze River. To gain a comprehensive view on the potential risk it would yet be necessary to extend the variety of investigated organic pollutants in biota for other important bioaccumulative toxicants, e.g., PCBs. They have been considered only in two studies, though they could be widely measured in water and sediment along the river. Moreover, besides organic components also heavy metals are known to accumulate in biota and therefore need to be integrated in an overall risk assessment. To summarize, prior to a risk of secondary intoxication for humans the detected organic contaminants pose a higher risk for adverse effects in the aquatic organisms themselves.
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1.5 Conclusion

Concerning the question if there is a solution of the pollution problem by dilution, we agree with the findings of Müller et al. (2008) that the Yangtze River’s immense amounts of water and sediment indeed reduce the ecotoxicological risk growing from pollution all along the river, but do not eliminate it. Though the largest share of measured pollutant levels in water was below Chinese national standards (Ministry of Environmental Protection - China 2002), ERL/ERM values (Long et al. 1995) suggested ecotoxicological impacts in certain areas, which could also be detected in vitro, in vivo and in situ. Hot-spot pollution of highly populated areas, as economic centers like Wuhan, revealed an immediate local impact. Further, as it can be seen from the example of the Yangtze Estuary, the particulate-bound pollution carried away from upstream sources can manifest in the downstream course and influence distant areas. The intrusion of saltwater at the river’s mouth causes a reduction in flow rate, which elevates the sedimentation rate and leads to an accumulation of particle bound pollutants. In addition, the increase in salinity can cause a shift in the pollutants affinity from water to sediment, as shown for PFOS. The consequence is that hydrophobic organic pollutants are trapped in the estuary. With respect to the high persistence of the detected compounds like PAHs, PCBs and OCPs, and the enduring production of emerging pollutants it is to be expected that the Yangtze Estuary will continue to suffer the pollution discharge of the river and that the contamination in this area will still increase in the future. Prevention including both direct and diffuse pollution control should therefore be the first choice, remediation the second. Countermeasures like the improvement of domestic and industrial wastewater treatment plants are recommended.

The water level’s influence on the concentrations and effects of pollutants in the investigated rivers could be shown in various studies. Those were generally higher during low water period in the dry season in winter/spring than during the rainy season in summer. This observation has been attributed to different hydrologic factors: on the one hand to the difference in amounts of discharged water and particulate matter, causing a greater or lesser dilution, and on the other hand to the reduced velocity during low water level. The lower flow rate causes an enrichment of pollutants in the water as well as higher sedimentation rates, which lead to the accumulation of particle bound pollutants in sediments. Due to the inverted water level situation in the TGR since impoundment, with high water level in winter and low water level in summer, it is to be expected that the changes in hydrologic conditions manifest as changes in the contamination status, with greater pollutant and effect levels during rainy season than during dry season.
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Hydrophilic compounds may be carried with the discharged water past the dam, but hydrophobic substances will most likely remain in the sediments of the reservoir. It has also to be considered that the elevated precipitation in summer is associated with an increased amount of air-borne particles washed out from the air as well as a stronger runoff from fields, carrying pollutants into the TGR, which may also be trapped there and subsequently accumulate in this region. To verify this assumption it is recommended to continuously monitor these changes.

Moreover it has to be considered that solely organic pollutants were in the scope of this review. Other impacts and risks on the Yangtze River’s biota and residents, e.g., growing from heavy metals or eutrophication due to high input of nitrates and phosphates from sewage and fertilizers as well as land use changes and the construction of dams that influence the river’s ecosystem, have to be kept in mind and included into an overall assessment.

The main research reflected by the number of reviewed publications was performed between Chongqing and the Yangtze Rivers mouth at the East China Sea, with particular focus on the areas of Chongqing, Wuhan, Nanjing, Shanghai and the Yangtze Estuary. Whereas the reaches upstream Chongqing were only little investigated. In comparison, the areas of main interest were also the main contaminated sites. This can be associated with an increasing population, industrialization and intensified agricultural usage from the Upper Reaches along the river’s downstream course to the Middle and Lower Reaches, with certain hot-spots in highly populated and industrial areas. Furthermore, despite its increasing importance only a small amount of published studies were available on the Three Gorges Reservoir after impoundment.

A striking observation was that many tributaries presented higher pollution levels and toxicity than the mainstream. This was in particular the case at some economic centers such as Chongqing (Jialing River) and Wuhan (Han River). The comparably lower levels in the mainstream can most likely be attributed to the vast amounts of water carried by it. The mainstream incorporates also less polluted tributaries and thereby dilutes the input and toxicity from higher polluted sources. This demonstrates the need to also monitor and initiate countermeasures at tributaries of the entire Yangtze River network, which are home to numerous people and habitat of a large variety of biota.

The largest part of the reviewed studies focused on pollutant levels in water and sediment, especially on PAHs, PCBs, OCPs, PCDDs/DFs, PBDEs, and PFCs. PAHs were the dominant pollutant class upon all contaminants detected in the river, predominantly sourcing from combustion of coal, wood and petroleum as well as from vehicle emission. The combustion of
coal in addition to wastewater discharges seemed to be the main contributors to PAH contamination in Chongqing section. The PAHs in sediments at Wuhan section were mainly caused by coal burning and petroleum combustion. Whereas PAHs found in Yangtze Estuary in the near-shore area mainly derived from petroleum and biomass (mainly coal) combustion, as well as vehicle emission; those detected in the farther shore zone originated mainly from petroleum combustion of shipping processes and shore side discharge. Chongming Island in Yangtze Estuary revealed PAHs from sewage as well as petroleum and coal combustion on the island itself. As an alluvial island, meaning formed by redeposited suspended matter, it is also influenced by upstream sources, because particle-bound PAHs carried by the water accumulate around the island. Furthermore, due to its special climate the south wind in summer carried PAHs in suspended particles from Shanghai city to Chongming Island.

Environmental quality standards for total PAHs are not applicable due to the complexity of this substance class (EWFD Directive 2000/60/EC, EWFD Directive 2008/105/EC). However, BaP can be used as an indicator for PAH contamination (ICPR 2009). The available studies revealed that BaP levels in water at Wuhan section, Jiangsu section exceeded the relevant standards given by the Ministry of Environmental Protection - China (2002) and also those of the European Water Framework Directive (EWFD Directive 2000/60/EC), an integrated river basin management to improve the quality of European water bodies. However, the concentrations of BaP in sediments along the River were all below the “relevant contamination” (> fourfold ICPR target value) set forth by the ICPR (2009), which is based on the EWFD (EWFD Directive 2000/60/EC, EWFD Directive 2008/105/EC). Based on the ERL and ERM guideline values (Long et al. 1995) maximum concentrations of total PAHs at Wuhan section and Yangtze Estuary rise concern for ecotoxicological impacts on aquatic life.

Measured PCB levels along the Yangtze River were also below a “relevant contamination” according to the ICPR classification (ICPR 2009). Yet the exceeded ERL guideline value (Long et al. 1995) indicates that the PCB levels in sediments at Wuhan section and the Yangtze Estuary may induce adverse effects. Attention should be paid to the release of PCBs as well as PBDEs into the environment from improper importation and disposal of e-waste along the river.

DDTs, HCHs and PCDDs/DFs residues in the Yangtze River have originated from the usage of pesticides in the past decades. DDT concentrations in all the sampling sections exceeded the ERL value (Long et al. 1995) suggesting a potential ecotoxicological risk in those areas. Attention should be paid to new input sources of DDTs and HCHs, e.g., by the usage of lindane and diconolf in some agricultural areas of the middle and lower Yangtze Reaches.
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Biological and chemical degradation can contribute to the reduction of organic pollutant levels in the environment in addition to dilution, and have to be considered as additional diminution factors for a broad variety of organic compounds. But most of the dominating substance classes that were reported by the reviewed articles shared the critical property persistence, which means that they are hardly degradable. Thus, degradation can only play a minor role for decreased concentrations of these compounds. Against the background of an immensely fast developing economy and considering the critical properties persistence and toxicity of pollutants like PAHs and PFCs it is to be expected that the pollution levels will rise in the future. Besides, though most of the contaminants are represented only in low levels at the TGR, except partially high PFOA concentrations, the organic pollutant distribution in sediments along the river has been influenced by the construction of TGD (Li et al. 2012a, Wang et al. 2012b), and require a long-term monitoring.

Among measurable ecotoxicological endpoints mutagenicity and endocrine activity were especially of predominant interest and widely detectable along the river. The mutagenicity in several sections of the Yangtze River and its tributaries can most likely be attributed to the widely distributed PAHs and nitro-PAHs, predominantly originating from combustion sources. Countermeasures to reduce the emission of PAHs into the environment are necessary, e.g., catalysts for cars, pollutant filter for factories and chiefly alternatives to fossil energy sources.

In several studies an increase in mutagenicity of tap water after drinking water treatment was shown and was most likely induced by disinfection byproducts, like THM, emerging from the chlorination of dissolved organic matter and other substances during the disinfection process. Thus, it is highly recommended to substitute the chlorination of polluted source water by less harmful water treatment methods for the preparation of drinking water.

Though some information, mainly on OCPs, PBDEs and PFCs in carp, catfish, mollusks and crabs were available, hardly any toxicological endpoints, except the formation of micronuclei in fish, were examined in situ. These studies, focusing on genotoxic effects, revealed that DNA damages occurred in fish from the Yangtze River, proving environmentally relevant concentrations of pollutants in the water. Wild fish and other aquatic organisms are an important dietary source in China. Marine algae and plants are considered to be nutritionally rich and wild fish are thought to be beneficial to human health. Therefore relatively large quantities of them are still consumed in China (Su et al. 2012). This means that contaminated fish, crabs, muscles and other aquatic organisms as well as contaminated drinking water bear a risk to residents benefiting from the river as a source for sustenance with respect to acute and chronic
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intoxication. However, the available articles revealed only a low risk of secondary intoxication with organic pollutants by consuming aquatic organisms from the Yangtze River. Prior to a risk of secondary intoxication for humans the detected organic contaminants posed a higher risk for adverse effects in the aquatic organisms themselves. To estimate the entire risk growing from bioaccumulation to the organisms as well as the consumers it is recommended to intensify the research on especially hazardous and well known bioaccumulative contaminants, like the ubiquitously detected PCBs in water and sediment as well as heavy metals.

Europe faced and still faces comparable issues as the Yangtze River basin. The Rhine, which is one of the economically, socially and environmentally most important rivers in Europe, and also an intensely examined and monitored water body, can serve as an example for future approaches concerning the Yangtze River. Though concentrations of organic pollutants may be lower in Yangtze River than in Rhine River, due to a comparably higher mass transport of water and particulate matter, it still results in comparably larger amounts of organic pollutants that end up in the Yangtze River’s receiving water body. The Yangtze River’s mean water discharge (30,200 m³/s) (National conditions - China 2003) is about fourteen times greater compared to the Rhine River (2,200 m³/s) (Huisman et al. 2000). This implicates that at same contamination levels in both rivers the fourteen fold amount of toxic substances would still enter the East China Sea. Therefore, the impact of the Yangtze River on the East China Sea needs to be taken into account.

Several approaches can help to preserve and improve the water quality of the Yangtze River: (1) The Yangtze should be seen in context of the River Continuum Concept (Vannote et al. 1980), an approach to describe and evaluate a river from its source to its estuary, considering it as an open and holistic ecosystem. It integrates biotic parameters, physical and hydrological factors, energy and nutrient input as well as output. This has also been applied for European river systems in the European Union Water Framework Directive (EWFD Directive 2000/60/EC). (2) Chemical/ecotoxicological and hydraulic approaches should be combined, e.g., to estimate the risk growing from remobilization of pollutants during flood events (Wölz et al. 2009, Brinkmann et al. 2010). This appears to be even more important compared to the Rhine River due to higher mass transport of water and sediment in the Yangtze River. (3) Bioassay-directed analysis should be applied to identify relevant toxic components (Hecker & Hollert 2009). (4) In situ investigations of mechanism-specific toxicity, like genotoxicity in blood cells from fish caught in the river, should serve as additional lines of evidence in Weight of Evidence studies, in order to prove the relevance of lab-based assays for the situation in the
field (Chapman & Hollert 2006, Boettcher et al. 2010). (5) Integrated assessments, like the triad approach (Chapman 1990), should be deployed to evaluate the relevance of pollutants for organisms and to gain a comprehensive view on the pollution status. At least, each effect assessment studied in vitro, in vivo or in situ should be accompanied by chemical analysis to identify possible inducers. (6) Toxicity reduction evaluation should be applied to areas with elevated toxicity levels. Advanced methods of wastewater treatment and integrated strategies to minimize the impact of point and non-point sources should be further developed - e.g., by transfer of knowledge from national research programs and applications (Huckele & Track 2013, Triebskorn et al. 2013) to bilateral and international joint projects in China (e.g., Bergmann et al. 2011 and Clean Water Initiative, China).

In conclusion, it is recommended to intensify the monitoring of the Yangtze River region and adjacent waters, as well as the implementation of countermeasures to reduce the emission of pollutants into this highly important water body to preserve its unique environment and the benefits growing from it for the Chinese people.
-Part 2-

Ecotoxicological impacts on the Three Gorges Reservoir

This chapter has been published as parts of two articles in peer-reviewed journals:


2 Ecotoxicological impacts on the Three Gorges Reservoir

2.1 Abstract

The construction of the Three Gorges Dam resulted beside its benefits also in detriments, like the inundation of cities and industrial sites, increasing urbanization, rising ship traffic and progressive industrialization, which trigger new pollution scenarios that have the potential to threaten the recently established Three Gorges Reservoir (TGR) ecosystem. These anthropogenic impacts might have serious consequences for the TGR’s biota and the people of the area that depend on this young and unique ecosystem.

Organic pollutants, particularly mutagens and genotoxicants, as well as aryl hydrocarbon receptor (AhR) agonists – like polycyclic aromatic hydrocarbons and other dioxin-like compounds -, have been widely detected in the Yangtze River, but only little research was yet done on the TGR area after impoundment, and AhR-mediated activities in particular. In order to record organic contamination and find links to ecotoxicological impacts, as well as to serve as reference for ensuing monitoring, several sites in the TGR area were screened applying the triad approach with additional lines of evidence. It combines chemical analysis, in vitro, in vivo and in situ investigations to a holistic assessment.

Sediments and the benthic fish species Pelteobagrus vachellii were sampled in 2011 and 2012, respectively, to identify relevant endpoints. (a) Sediment was analyzed for 54 relevant organic compounds based on the European Water Framework Directive and (b) tested in vitro with the Ames fluctuation assay for mutagenicity, as well as (c) the ethoxyresorufin-O-deethylase (EROD) induction assay for AhR-mediated activity. Further, (d) the sediment was investigated in vivo with the Fish Embryo Toxicity Test (extractable fraction) and (e) Sediment Contact Assay (bioavailable fraction) with Danio rerio to both test for embryotoxicity/teratogenicity. In situ studies with P. vachellii comprised (f) the quantification of biliary pollutant metabolites and (g) micronucleus formation in erythrocytes to assess genotoxic impacts. In addition, activities of hepatic (h) phase I (EROD) and (i) phase II (glutathione S-transferase) biotransformation enzymes were measured in situ to both determine AhR-mediated activities. Further, histopathological alterations in liver and excretory kidney of P. vachellii were evaluated, inter alia to assess immunotoxic impacts. EROD induction was tested in vitro and in situ to evaluate possible relationships between the activity of sediments and effects determined in the fish. Two sites, near Chongqing and Kaixian city, were identified as regional
hot-spots and further investigated in 2013. Sediment and fish samples were taken in parallel at the hot-spots and analyzed with a set of relevant endpoints.

Only polycyclic aromatic hydrocarbons (PAHs) could be detected in sediments from 2011 (165-1,653 ng/g), emphasizing their role as key pollutants of the area. Their ubiquity was confirmed at Chongqing (150-433 ng/g) and Kaixian (127-590 ng/g) in 2013. Concentrations were comparable to other major Chinese and German rivers. However, the immense sediment influx suggests a deposition of 216-636 kg PAH/day (0.2-0.6 mg PAH/m²/day), indicating an ecotoxicological risk. PAH source analysis highlighted the primary impact of combustion sources on the more industrialized upper TGR section, whereas petrogenic sources dominated the mid-low section.

Furthermore, sediment extracts from several sites exhibited significant impacts of frameshift promutagens in the Ames fluctuation assay. The sediments induced in the in vitro/in vivo bioassays AhR-mediated activities and embryotoxic/teratogenic effects – particularly on the cardiovascular system. These endpoints could be significantly correlated to each other and respective chemical data. However, particle-bound pollutants showed only low bioavailability.

The in situ investigations suggested a rather poor condition of *P. vachellii*, with histopathological alterations in liver and excretory kidney. Significant genotoxic impairments in erythrocytes of *P. vachellii* were detected (Chongqing/Kaixian), demonstrating the relevance of genotoxicity as an important mode of action in the TGR’s fish. In addition, fish from Chongqing city exhibited significant hepatic EROD induction and obvious parasitic infestations. The PAH metabolite 1-hydroxypyrene was detected in bile of fish from all sites.

All endpoints in combination with the chemical data suggest a pivotal role of PAHs in the observed ecotoxicological impacts. PAHs, their derivatives and non-target compounds are considered as main causative agents.
2.2 Triad A: Chemistry

2.2.1 Total PAH content

In the sampling campaign during September 2011, among all 54 analyzed compounds (Table II-2) only the 16 priority PAHs could be detected (Table III-2.1). The total PAH (PAH$_{216}$) concentrations ranged from 165 ng/g (BJX-R/reference site) to 1,653 ng/g (CNG-U).

Each upstream sample of the four sites along the mainstream displayed the highest PAH$_{216}$ content – with exception of Yunyang – and the tributaries the lowest, giving the dominating pattern for PAH$_{216}$ content per site: upstream > downstream > tributary. Inter-site comparison along the mainstream, integrating each three samples per site, showed the following order for PAH$_{216}$ content: Yunyang > Chongqing > Wushan > Fengdu (Table III-2.1). Further, it could be observed that the PAH$_{216}$ content from the downstream location of one site typically increased to the upstream location of the next site in flow direction (e.g., CNG-D to FEN-U) – with exception of YUN-D/WU-U (Table III-2.1), which could hint to an accumulation of PAH contamination between the tributaries, due to lesser dilution.

In sampling campaign May 2013 PAH$_{216}$ concentrations ranged from 150 ng/g (TGR-B) to 433 ng/g (YAN-C) at Chongqing, and 127 ng/g (HAN-B) to 590 ng/g (HAN-C) at Kaixian (Table III-2.2). In general a decline in the PAH$_{216}$ concentration could be measured in the sediments along the flow direction - with exception of YAN-C. The concluding pattern was YAN-C > YAN-A > YAN-B > TGR-A > TGR-B for the Yangtze River and JIA-A > JIA-B > JIA-C > TGR-A for the Jialing River. In comparison the Yangtze River displayed a higher PAH$_{216}$ content than the Jialing River and the TGR (Table III-2.1; Table III-2.2). These values correspond to a study by Tang et al. (2011), who found comparable PAH$_{216}$ concentrations in sediments of the Yangtze River (257-723 ng/g) and Jialing River (132-349 ng/g) sampled at Chongqing in October 2009.

At Kaixian, HAN-D and HAN-C possessed a three to four times higher PAH$_{216}$ concentration than HAN-B and HAN-A (Table III-2.2). This indicates a pollutant influx between HAN-B and HAN-C, which could originate from the city of Kaixian on the south side and/or the Dong or Toudao River, which enter the lake on the north side. This assumption is underlined by the observation that benzo[b]fluoranthene was alike benzo[k]fluoranthene below the LOD in the upper part of the Hanfeng Lake (HAN-A, HAN-B), but both could be measured in the lower part of the Hanfeng Lake (HAN-C, HAN-D) (Table III-2.2).
Table III-2.1. PAH content in sediment extracts of sampling campaign September 2011 (ng/g)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Chongqing</th>
<th>Fengdu</th>
<th>Yunyang</th>
<th>Wushan</th>
<th>Kaixian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNG -U</td>
<td>CNG -T</td>
<td>CNG -D</td>
<td>YUN -U</td>
<td>YUN -T</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>360</td>
<td>220*</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>12</td>
<td>12</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td>Acenaphthene</td>
<td>99</td>
<td>33*</td>
<td>&lt;10</td>
<td>14</td>
<td>&lt;10</td>
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<tr>
<td>Fluorene</td>
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<td>64*</td>
<td>21*</td>
<td>20*</td>
<td>&lt;10</td>
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<tr>
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<td>340*</td>
<td>120</td>
<td>130</td>
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<tr>
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<td>29</td>
</tr>
<tr>
<td>Fluoranthenea</td>
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<tr>
<td>Pyrene</td>
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<td>150</td>
<td>47</td>
<td>99</td>
<td>100</td>
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<tr>
<td>Benzo[a]anthracenea,b,c</td>
<td>605</td>
<td>73</td>
<td>19</td>
<td>64</td>
<td>62</td>
</tr>
<tr>
<td>Chrysenea,b,c</td>
<td>1695</td>
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<td>41</td>
<td>65</td>
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<td>120</td>
</tr>
<tr>
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<td>38</td>
<td>12</td>
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<td>28</td>
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<tr>
<td>Benzo[a]pyrenea,b,c</td>
<td>626</td>
<td>76</td>
<td>21</td>
<td>57</td>
<td>52</td>
</tr>
<tr>
<td>Indeno[1,2,3-c,d]pyrenea,b,c</td>
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<td>57</td>
<td>21</td>
<td>41</td>
<td>44</td>
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<tr>
<td>Dibenzo[a,h]anthracenea,b,c</td>
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<td>Benzo[g,h,i]perylene</td>
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<td>Σ PAHs</td>
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<td>816</td>
<td>1122</td>
<td>310</td>
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<tr>
<td>Σ PAHs w/ Ames activity</td>
<td>717</td>
<td>223</td>
<td>482</td>
<td>514</td>
<td>160</td>
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<tr>
<td>Σ PAHs w/ EROD activity</td>
<td>547</td>
<td>168</td>
<td>362</td>
<td>394</td>
<td>122</td>
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<tr>
<td>TOC mean [%]</td>
<td>1.65</td>
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<td>0.40</td>
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</tr>
<tr>
<td>TOC SD [%]</td>
<td>0.02</td>
<td>0.04</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* = Substance has been shown to be genotoxic in the Ames assay (Pérez et al. 2003); ** = Substance is carcinogenic according to WHO (2010); *** = Substance has been shown to induce EROD activity in RTL-W1 cells (Bols et al. 1999); # = concentration exceeded „effect range low“ value according to Long et al. (1995) (Table III-2.5); TOC = Total organic carbon; SD = Standard deviation; chemical analysis n = 1; TOC analysis n = 3.
<table>
<thead>
<tr>
<th>Substance [ng/g]</th>
<th>Σ substance</th>
<th>Chongqing</th>
<th>Yangze</th>
<th>Jialing</th>
<th>TGR</th>
<th>Kaixian</th>
<th>Hanfeng Lake</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>YAN -A</td>
<td>YAN -B</td>
<td>YAN -C</td>
<td>JIA -A</td>
<td>JIA -B</td>
<td>JIA -C</td>
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<td>35</td>
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<td>38</td>
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<tr>
<td>Acenaphthylene</td>
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<td>16</td>
<td>17</td>
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<td>18</td>
<td>16</td>
</tr>
<tr>
<td>Acenaphthene</td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Fluorene</td>
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<td>14</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>19*</td>
<td>15</td>
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<tr>
<td>Phenanthrene</td>
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<td>29</td>
<td>42</td>
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<td>22</td>
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<tr>
<td>Fluoranthene(^a)</td>
<td>442</td>
<td>63</td>
<td>43</td>
<td>57</td>
<td>40</td>
<td>35</td>
<td>35</td>
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<tr>
<td>Pyrene</td>
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<td>25</td>
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<td>41</td>
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<td>22</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene(^a,b,c)</td>
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<td>31</td>
<td>27</td>
<td>44</td>
<td>13</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene(^a,b,c)</td>
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<td>22</td>
<td>25</td>
</tr>
<tr>
<td>Benzo[a]pyrene(^a,b,c)</td>
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<td>Indeno[1,2,3-c,d]pyrene(^a,b,c)</td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene(^a,b,c)</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
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<tr>
<td>Benzo[g,h,i]perylene</td>
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<td>&lt;10</td>
<td>&lt;10</td>
<td>18</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Σ PAHs</td>
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<td>297</td>
<td>433</td>
<td>299</td>
<td>291</td>
<td>278</td>
<td>254</td>
</tr>
<tr>
<td>Σ PAHs w/ Ames activity</td>
<td>226</td>
<td>150</td>
<td>232</td>
<td>117</td>
<td>115</td>
<td>141</td>
<td>137</td>
</tr>
<tr>
<td>Σ PAHs w/ EROD activity</td>
<td>163</td>
<td>107</td>
<td>176</td>
<td>77</td>
<td>80</td>
<td>107</td>
<td>98</td>
</tr>
</tbody>
</table>

| TOC mean [%]    | 0.26    | 0.39   | 0.36   | 0.45   | 0.66  | 0.30   | 0.19   | 0.11   | 0.12  | 0.27  | 1.00  | 0.99   |
| TOC SD [%]      | 0.08    | 0.03   | 0.05   | 0.05   | 0.10  | 0.03   | 0.01   | 0.01   | 0.02  | 0.03  | 0.04  | 0.01   |

\(^a\) = Substance has been shown to be mutagenic in the Ames assay (Pérez et al. 2003); \(^b\) = Substance is cancerogenic according to WHO (2010); \(^c\) = Substance has been shown to induce EROD activity in RTL-W1 cells (Bols et al. 1999); \(^*\) = concentration exceeded "effect range low" value according to Long et al. (1995) (Table III-2.5); TOC = Total organic carbon; SD = Standard deviation; chemical analysis n = 1; TOC analysis n = 3.
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The temporal variation between the campaigns in September 2011 and May 2013 showed that PAH concentrations were generally higher in September 2011. These observations stand in agreement with Wang et al. (2013), who referred the temporal differences in pollution to the drawdown period of the TGR (around May), where water quality is better than during the other seasons.

With regard to PAH$_{z16}$ concentrations in the water phase Wang et al. (2009) could measure overall 14 to 97 ng/L at sites along the reservoir - with highest concentrations at Chongqing, Changshou and Maoping - in May 2008. Overall, they stated that obvious regional variations of PAHs, PCBs and organochlorine pesticide levels appeared along the reservoir and that its water could be classified as being polluted by HCB and PAH based on water quality criteria (Wang et al. 2009). They repeated their investigations along the TGR in 2009 and 2011 and could detect PAH$_{z16}$ concentrations from 15 to 381 ng/L in surface water – with highest concentrations at Chongqing – and an obvious decrease from the upper to the lower part of the reservoir. Further, they found a dynamic relation of the contamination to anthropogenic activities and a certain contribution of the tributaries to the PAH pollution of the mainstream (Wang et al. 2013). Overall they could calculate a total PAH mass flux from 110 to 2,160 mg/sec (Deyerling et al. 2014). Wolf et al. (2013a,b) could determine PAH concentrations - Naphthalene excluded - between 5 to 101 ng/L (mean: 22 ± 33 ng/L) along the TGR between Chongqing and Yunyang, and also at the Hanfeng Lake in September 2011. The detected concentrations were designated to be in comparable ranges or even lower than measured in surface waters in western industrialized countries. Furthermore, concentrations of perfluorinated compounds, polychlorinated biphenyls and polybrominated diphenyl ethers, were below the respective detection limits, which was referred to their low solubility (Wolf et al. 2013a). The analyzed organic pollutants, with exception of the two pesticides Picloram and Clopyralid, met the standards for the Chinese National Drinking Water Quality Standard GB 5749 (Ministry of Health - China 2006) and the European Union (EU) Council Directive 98/83/EC on the quality of water intended for human consumption (The Council of the European Union 1998) and the EU Directive 2008/105/EC on environmental quality standards in the field of water policy (EWFD Directive 2008/105/EC, Wolf et al. 2013a).

In comparison to other sections of the Yangtze River the sampled locations in this study exhibited a similar PAH$_{z16}$ content, but were mostly rather in the lower part of the ranges detected at other sections of the river (Feng et al. 2007a, Wang et al. 2012b, Liu et al. 2014). Other Chinese rivers, like the Pearl River (Mai et al. 2002) and Yellow River (Yu et al. 2009),
exhibited also comparable and mainly higher PAH$_{16}$ concentrations. Also in international comparison the total PAH$_{16}$ content of TGR surface sediments were below or at the lower limit of, e.g. German main water bodies like Danube River (Keiter et al. 2008) and Rhine River - benzo[e]pyrene was analyzed instead of benzo[a]pyrene (Kosmehl et al. 2004).

Considering the mass balance for PAHs, under assumption that annually 151-172 Mt of sediment remain in the TGR (2003-2008) (Yang et al. 2007, Hu et al. 2009a) and that the average PAH$_{16}$ burden in sediment along the reservoir is 936 ± 414 ng/g (September 2011 campaign), an amount of about 79-232 t PAH$_{16}$ per year or 216-636 kg PAH$_{16}$ per day are deposited in the reservoir. Further, adopting the reservoirs surface area of 1080 km$^2$ as potential bottom deposition area of the reservoir (water body pictured as a cuboid) this results into about 0.2 - 0.6 kg PAH/km$^2$/day (0.2 - 0.6 mg PAH/m$^2$/day). This assumption neglects seasonal differences and less industrialized regions between the major tributaries, but also that an even larger portion of PAHs enters the reservoir, remains in the water phase and is discharged - solved in water or bound to particles - behind the dam. These numbers are in agreement with the mass balance estimation of Müller et al. (2008) that about 500 to 3,500 kg of phenols, chlorinated compounds, aromatic hydrocarbons and PAHs are discharged by the Yangtze River per day. Zhang and Tao (2009) estimated an annual atmospheric emission of 114,000 t PAH$_{16}$ for China in 2004 – accounting for 22% of the global emission, followed by India (17%) and the United States (6%).

2.2.2 Specific PAH content

The samples from campaign September 2011 have shown that overall the low molecular weight (LMW; 2-3 rings) PAH phenanthrene, the medium molecular weight (MMW; 4 rings) PAHs chrysene, fluoranthene and pyrene, as well as the high molecular weight (HMW; 5-6 rings) PAH benzo[h]fluoranthene formed the largest share of the total PAH spectrum among all samples, generally constituting together more than 70% of the total content (Table III-2.1).

Further, in sampling campaign May 2013 overall the LMW PAH naphthalene (13%) and the MMW PAH fluoranthene and pyrene were the most abundant PAHs at Chongqing, whereas at Hanfeng Lake the LMW PAH naphthalene and phenanthrene, the MMW PAH chrysene and the HMW PAH benzo[h]fluoranthene dominated the spectrum (Table III-2.2).

In TGR surface water Wang et al. (2009) found the LMW and MMW PAHs phenanthrene, fluoranthene, pyrene and chrysene to be the predominant PAHs in surface water of the TGR. This was confirmed by Wolf et al. (2013a), who stated that LMW and MMW PAHs were
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ubiquitously distributed, whereas the HMW PAHs could not be detected. They concluded that either atmospheric deposition of the more volatile LMW PAH, or a strong adsorption of the HMW PAHs to suspended particulate matter in the water column led to this distribution. Based on the observations of this study, that also LMW and MMW PAHs dominated the spectrum and less HMW PAHs were adsorbed to the sediment, a high atmospheric deposition of the more volatile PAHs is suggested.

2.2.3 Total organic carbon analysis

The TOC content ranged from 0.40 ± 0.01% (CNG-D) to 1.65 ± 0.02% (CNG-U) in campaign September 2011 (Table III-2.1), and from 0.11 ± 0.01% (TGR-B) to 0.66 ± 0.10% (JIA-B) at Chongqing, as well as 0.12 ± 0.02% (HAN-A) to 1.00 ± 0.04% (HANC) at Kaixian in May 2013 (Table III-2.2). In general, the PAH216 did not correspond to the TOC content per sample. However, the rise in TOC corresponded to the increase of PAH216 at Hanfeng Lake in May 2013 from 0.27 ± 0.03% (HAN-B) to 1.00 ± 0.04% (HAN-C), which emphasizes a pollutant influx between these sampling locations, particularly indicating the discharge of wastewater.

2.2.4 Principal Component Analysis

The chemical analysis of water/sediment samples from the Yangtze River and its tributaries targeting on PAH-compounds revealed a complex pattern of contents and identities. Quantitative analysis of the PAH content in sediment extracts from September 2011 (Fig. III-2.1) resulted in two principal key factors that together explain 74% of the total variance of the dataset. Obvious is the separation of MMW PAHs (4 aromatic rings: FLA, PYR, BaA, CHR) to HMW PAHs (5-6 rings: BbF, BkF, BaP, IcdP, DahA, BghiP) in one group and LMW PAHs (2-3 rings: FL, ANT, NP, ACY, ACE) in a second group, with exception of phenanthrene that forms a group with the 4-6 ring PAHs. That demonstrates a differentiation of the sites, e.g., by the abundance or absence of low molecular PAHs of the second group (positive 2nd axis). Especially, the sites in the upper part of the TGR (CNG-U, CNG-D; FEN-U, FEN-D) including their tributaries (CNG-T; FEN-T) and the Hanfeng Lake (HF-L) at Kaixian are defined by the content of low molecular PAHs (Fig. III-2.1). Organizing all upstream, downstream and tributary samples in individual groups reveals an orientation of these groups along a gradient dominated by the MMW to HMW PAHs: upstream > downstream > tributaries. This indicates that the downstream locations are primarily influenced by the upstream locations, with possible dilution effects of the tributaries (Fig. III-2.1) – under assumption of a downstream oriented flow direction.
Fig. III-2.1. Principal component analysis of PAH concentrations (ng/g) in sediment extracts from sampling campaign September 2011 analyzed as quantitative data. Arrows resemble individual PAHs; length of arrows equals relative amount of PAHs; 1st axis: eigenvalue 0.48; 2nd axis: eigenvalue 0.26.

An intra-site comparison enables a more specific breakdown of the relationships between the local samples. Whereas the correlation between the downstream locations at Chongqing and Fengdu with the corresponding upstream locations (CNG-U and FEN-U, respectively) and tributaries (CNG-T and FEN-T, respectively) appear to be comparable, the Pengxi River (YUN-T) seems to have a stronger impact on the PAH composition of the downstream location (YUN-D) than the upstream location (YUN-U) at Yunya, as the datapoints of YUN-T and YUN-D overlap indicating a similar PAH pattern. However, the downstream location (WU-D) at Wushan appears to have a closer relationship with the upstream location (WU-U), than the Daning River (WU-T) (Fig. III-2.1).
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This could be explained by discharge boundary conditions in the conversion zone as mentioned by Holbach et al. (2013) for the Daning River and TGR mainstream. Furthermore, they observed a reversion of the Daning River’s flow direction, forced by the increasing water level of the Yangtze River mainstream in August 2011. This means that the Daning River was rather influenced by suspended particulate matter that was transported from the TGR into the less turbulent end section of the tributary. A similar observation could be made at the Xiangxi tributary, in the lower part of the TGR, in September 2012 (Holbach et al. 2014). Thus, it is reasonable to question if the tributaries influence the mainstream of the reservoir or if the situation is reversed. However, the relationship between Pengxi River (YUN-T) and the downstream location at Yunyang (YUN-D) could represent an exception of this situation.

Fig. III-2.2. Principal component analysis of PAH concentrations (ng/g) in sediment extracts from sampling campaign May 2013 analyzed as quantitative data. Arrows resemble individual PAHs; length of arrows equals relative amount of PAHs; 1st axis: eigenvalue 0.67; 2nd axis: eigenvalue 0.14.
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The two most important axes of the PCA using PAH content data in sediments from May 2013 explain 81% of the total variance of the dataset (Fig. III-2.2). The scenario is less differentiated than in September 2011. The distribution is particularly oriented at the abundance of naphthalene (NP), fluoranthene (FL), benzo[a]pyrene (BaP), acenaphthene (ACE) and benzo[g,h,i]perylene (BghiP). Considering the proportion of the 1st axis (67%) to the total variance, the samples from Chongqing show a large similarity, with exception of TGR-B, which exhibits the lowest content of PAH$_{216}$ at this location. The samples YAN-A, YAN-C and JIA-C strike out due to their content of benzo[a]pyrene (19 to 25 ng/g), which could not be detected above the LOD of 10 ng/g in any of the other samples (Fig. III-2.2; Table III-2.2). It could be a hint for potential input sources particularly close before the conversion zone between Yangtze and Jialing River (YAN-C, JIA-C); although it has to be considered that the difference to the LOD was only 9 to 15 ng/g. Benzo[a]pyrene could not be measured above the LOD at YAN-B, although it was detectable upstream and downstream of this location, and at TGR-B the lowest PAH$_{216}$ content of the area was detected (59% compared to TGR-A). This may emphasize the assumption that the pollution sources have a rather limited regional impact concerning particle affine contaminants. This is most likely due to the low flow velocity of the water bodies, which causes a higher sedimentation rate of suspended particulate matter, which can carry adherent contaminants. A similar observation could be made at Kaixian. At the Hanfeng Lake a large similarity between the samples from the upper part (HAN-A; HAN-B) could be found, as well as between the samples from the lower part (HAN-C; HAN-D), while the quantity and composition of the PAHs between those parts appear to be largely different. This can be most likely referred to pollution sources between HAN-B and HAN-C (cf chapter 2.1.1.). However, although HAN-A and HAN-B show comparable PAH$_{216}$ concentrations, the content increases to HAN-C and then decreases again to HAN-D (82% of HAN-C) (Table III-2.2), also showing a decline of PAH$_{216}$ contamination with the flow direction in the lower part of the lake (HAN-C > HAN-D).

2.2.5 PAH source analysis

Anthropogenic sources are the main PAH contributors in urbanized and industrialized regions, and can be divided into two groups. Petrogenic sources relate to crude oil, coal and their products, mainly released by spillage. While pyrogenic sources relate to incomplete combustion of biomass, like wood burning, and fossil fuels (Neff 1979, Mostert et al. 2010). Because PAHs are always emitted as a mixture and different emission sources are considered to have characteristic relative PAH ratios, it is possible to trace the contamination in environmental
samples (sediment, soil, water, air and tissue) - with some restrictions - back to their sources (Yunker et al. 2002, Tobiszewski & Namieśnik 2012). As emitted PAHs undergo various fate processes, which depend on factors like solubility and adsorption, typically substances of the same molecular mass and similar physicochemical properties are compared to minimize differences due to diverging transformations (Readman et al. 1987, McVeety & Hites 1988).

The important role of liquid fossil fuel combustion all along the reservoir (Table III-2.3) stands in agreement with results from the TGR water column by Wang et al. (2009, 2013). Urban traffic emissions and runoff, as well as intensified shipping activities since the impoundment of the reservoir, can be accounted to be the main contributors. Moreover, combustion sources had a major impact on the upper - highly urbanized and industrialized - part of the TGR (Table III-2.3; Table III-2.4), which is also verified by the water analysis by Wang et al. (2009, 2013). Particularly the cities of Chongqing and Changshou are important industrial centers at the TGR’s shore, where industrial emission – e.g., power generation from coal combustion - and urban air pollution, may have a serious influence on the contamination status of the reservoir in this region. On the other hand, the origin of PAHs in the middle (YUN) and lower part (WU) of the TGR rather could be traced back to petrogenic sources - in accordance with Wang et al. (2013) -, and upon combustion particularly to vehicular emission from traffic (Table III-2.3). Alike all tributaries – with exception of CNG-T -, which were also affected by petrogenic sources, this may be caused by oil and fuel spillage, e.g., from ships (Table III-2.3). As the downstream location at Yunyang (YUN-D) stood out to be seriously influenced by a contamination source, the origin appears to be petrogenic (Table III-2.3). The contamination at the Hanfeng Lake exhibited a mixture of petrogenic sources and combustion, especially from liquid fossil fuels as well as coal, grass and wood. This may be caused on the one hand side from urban air pollution, and on the other hand from biomass burning in homes as the city is located in a rather rural area. The lower locations at the Hanfeng Lake, that showed an increased contamination, were defined by petrogenic sources, which may originate from shipping activities and wastewater (Table III-2.3; Table III-2.4). The reference site (BJX-R) showed a dominating pyrogenic impact, with a mixture of liquid fossil fuel and also coal, grass and wood combustion. This can also be referred to biomass burning in homes of the rural area and minor shipping activities in the area (Table III-2.3). With regard to overall China, particularly biomass burning, domestic coal combustion and coke ovens have been identified as significant sources for the atmospheric emission of PAHs (Zhang et al. 2007, Zhang & Tao 2009).
Table III-2.3. Diagnostic ratios for PAHs in sediment from campaign September 2011 with related sources.

<table>
<thead>
<tr>
<th>PAH diagnostic ratios</th>
<th>Chongqing</th>
<th>Fengdu</th>
<th>Yunyang</th>
<th>Wushan</th>
<th>Kaixian</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CNG-U</td>
<td>CNG-T</td>
<td>CNG-D</td>
<td>FEN-U</td>
<td>FEN-T</td>
</tr>
<tr>
<td>ANT/ANT+PHE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11</td>
<td>0.11</td>
<td>0.17</td>
<td>0.11</td>
<td>&lt;0.11&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>FLA/FLA+PYR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53</td>
<td>0.54</td>
<td>0.55</td>
<td>0.54</td>
<td>0.53</td>
</tr>
<tr>
<td>BaA/BaA+CHR&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.38</td>
<td>0.31</td>
<td>0.49</td>
<td>0.44</td>
<td>0.34</td>
</tr>
<tr>
<td>LcdP/LcdP+BghiP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.43</td>
<td>0.45</td>
<td>0.48</td>
<td>0.44</td>
<td>0.44</td>
</tr>
</tbody>
</table>

<sup>a</sup> ANT = Anthracene, PHE = Phenanthrene, Source: <0.1 = petroleum, >0.1 = combustion (Pies et al. 2008);<sup>b</sup> FLA = Fluoranthene, PYR = Pyrene, Source: <0.4 = petroleum, 0.4-0.5 = vehicular emission, >0.5 = grass/wood/coal combustion (De la Torre-Roche et al. 2009);<sup>c</sup> BaA = Benzo[a]anthracene, CHR = Chrysene, Source: <0.2 = petroleum, 0.2-0.35 = petroleum/combustion, >0.35 = combustion (Yunker et al. 2002);<sup>d</sup> LcdP = Indeno[1,2,3-c,d]pyrene, BghiP = Benzo[g,h,i]perylene, Source: <0.2 = petroleum, 0.2-0.5 = liquid fossil fuel combustion (vehicle/crude oil), >0.5 = grass/wood/coal combustion (Yunker et al. 2002);<sup>e</sup> Concentration of anthracene was <10 ng/g;<sup>f</sup> Concentration of Indeno[1,2,3-c,d]pyrene was <10 ng/g.

Table III-2.4. Diagnostic ratios for PAHs in sediment from campaign May 2013 with related sources.

<table>
<thead>
<tr>
<th>PAH diagnostic ratios</th>
<th>Yangtze</th>
<th>Chongqing</th>
<th>Jialing</th>
<th>TGR</th>
<th>Kaixian</th>
<th>Hanfeng Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YAN-A</td>
<td>YAN-B</td>
<td>YAN-C</td>
<td>JIA-A</td>
<td>JIA-B</td>
<td>JIA-C</td>
</tr>
<tr>
<td>ANT/ANT+PHE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37</td>
<td>0.38</td>
<td>0.31</td>
<td>0.34</td>
<td>0.36</td>
<td>0.44</td>
</tr>
<tr>
<td>FLA/FLA+PYR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53</td>
<td>0.55</td>
<td>0.56</td>
<td>0.54</td>
<td>0.53</td>
<td>0.54</td>
</tr>
<tr>
<td>BaA/BaA+CHR&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.37</td>
<td>0.47</td>
<td>0.43</td>
<td>0.51</td>
<td>0.51</td>
<td>0.54</td>
</tr>
<tr>
<td>LcdP/LcdP+BghiP&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.53&lt;sup&gt;g&lt;/sup&gt;</td>
<td>n.d.</td>
<td>&lt;0.36&lt;sup&gt;g&lt;/sup&gt;</td>
<td>n.d.</td>
<td>n.d.</td>
<td>&lt;0.59&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> ANT = Anthracene, PHE = Phenanthrene, Source: <0.1 = petroleum, >0.1 = combustion (Pies et al. 2008);<sup>b</sup> FLA = Fluoranthene, PYR = Pyrene, Source: <0.4 = petroleum, 0.4-0.5 = vehicular emission, >0.5 = grass/wood/coal combustion (De la Torre-Roche et al. 2009);<sup>c</sup> BaA = Benzo[a]anthracene, CHR = Chrysene, Source: <0.2 = petroleum, 0.2-0.35 = petroleum/combustion, >0.35 = combustion (Yunker et al. 2002);<sup>d</sup> LcdP = Indeno[1,2,3-c,d]pyrene, BghiP = Benzo[g,h,i]perylene, Source: <0.2 = petroleum, 0.2-0.5 = liquid fossil fuel combustion (vehicle/crude oil), >0.5 = grass/wood/coal combustion (Yunker et al. 2002);<sup>g</sup> n.d. = no diagnostic ratio could be determined.
2.3 Triad B: *In vitro/in vivo* bioassays

2.3.1 Assessment of biological adverse effects according to sediment quality guidelines

PAHs have been classified as persistent organic pollutants, due to their (semi-)persistent, bioaccumulative and toxic properties. Upon those are the 16 analyzed PAHs *(Table II-2)* listed as priority pollutants by the U.S. Environmental Protection Agency (1982). PAHs can be found throughout all environmental media – air, soil, water, sediment, tissue – and exhibit impairing properties, for example cancerogenicity and mutagenicity (Pérez et al. 2003, WHO 2010). Due to the complexity of PAH mixtures and their individual effects no official standards exist so far - only for indicator compounds like BaP - to evaluate the biological effects of total PAHs in sediment. However, one attempt to set evaluation values in order to anticipate biological effects is the ERL/ERM concept (“effect range low”/“effect range median”) by Long et al. (1995). These values delineate three ranges in substance concentrations that are associated with (a) rarely (<ERL), (b) occasionally (ERL≥ and <ERM) and (c) frequently (≥ERM) occurring effects (Long et al. 1995). Effect range values exist for individual PAHs and total PAHs for which the correlation between effect and substance concentration have been noted to be good *(Table III-2.5)* (Long et al. 1995). Overall no sample exceeded the ERL value for total PAHs (4,022 ng/g dry weight [dw]), thus suggesting that rarely biological effects were to be expected *(Table III-2.1; Table III-2.2)*. However, most samples exceeded the ERL values for one or more individual PAHs, but none the corresponding ERM values, thus indicating occasional biological impairments. Particularly, the low molecular weight PAHs fluorene, phenanthrene, acenaphthene and naphthalene were problematic. The according samples were CNG-U, CNG-T, CNG-D, FEN-U, FEN-D, YUN-U, YUN-T, YUN-D, WU-U, HF-L, JIA-B, HAN-C and HAN-D.
Table III-2.5. Effect range low (ERL) and effect range median (ERM) guideline values for PAHs (ng/g) according to Long et al. (1995).

<table>
<thead>
<tr>
<th>Compound</th>
<th>ERL</th>
<th>ERM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>160</td>
<td>2100</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>44</td>
<td>640</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>16</td>
<td>500</td>
</tr>
<tr>
<td>Fluorene</td>
<td>19</td>
<td>540</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>240</td>
<td>1500</td>
</tr>
<tr>
<td>Anthracene</td>
<td>85.3</td>
<td>1100</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>600</td>
<td>5100</td>
</tr>
<tr>
<td>Pyrene</td>
<td>665</td>
<td>2600</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>261</td>
<td>1600</td>
</tr>
<tr>
<td>Chrysene</td>
<td>384</td>
<td>2800</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>430</td>
<td>1600</td>
</tr>
<tr>
<td>Indeno[1,2,3-c,d]pyrene</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>63.4</td>
<td>260</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>LMW PAH</td>
<td>552</td>
<td>3160</td>
</tr>
<tr>
<td>MMW + HMW PAH</td>
<td>1700</td>
<td>9600</td>
</tr>
<tr>
<td>Σ PAH</td>
<td>4022</td>
<td>44792</td>
</tr>
</tbody>
</table>

n.a. = not available; *a no safety values exist as it is suggested that merely presence causes toxic effects with high probability (Feng et al. 2007a, Liu et al. 2009).

2.3.2 Ames fluctuation assay

For the samples from campaign September 2011 the exposure of bacteria tester strain TA100 in the presence and absence of exogenous enzymatic S9 supplement to the sediment extracts resulted in no significant mutagenic effects ($p>0.05$), with exception of YUN-D with metabolic activation (NOEC: 200 mg/mL; $p<0.05$). The same procedure with the tester strain TA98 in the absence of S9 supplement also led to no significant results ($p>0.05$), with exception of CNG-D (NOEC: 200 mg/mL; $p<0.05$). However, the exposure of tester strain TA98 in presence of S9 supplement resulted in several significant mutagenic activities ($p<0.05$) (Table III-2.6). The strongest inducing samples were CNG-T, FEN-D, WU-U and WU-D with a NOEC of 25 mg/mL ($p<0.05$), while FEN-U, WU-T, HF-L and the reference site BJX-R exhibited no significant impact at all (NOEC: >400 mg/mL; $p>0.05$) (Table III-2.6). Testing of the sediment extracts from the May 2013 sampling with bacteria tester strain TA98 in absence of metabolic activation revealed no significant mutagenicity in any of the samples ($p>0.05$). Also,
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Adding of S9 supplement resulted only in a significant effect in the samples HAN-D (NOEC: 100 mg/mL) and HAN-A (NOEC: 200 mg/mL) \( (p<0.05) \). However, cytotoxicity was observed for the extracts of JIA-A, JIA-B and HAN-C (400 mg/mL), which might explain the absence of mutagenicity in these samples.

Potential sources for the mutagenic compounds in the strongest inducing samples (25 mg/mL) may be referred to local industries. The mutagenicity at CNG-T and the cytotoxicity found in JIA-A and JIA-B emphasize an industrial impact on the Jialing River, where several industrial sites have settled. At Fengdu, the downstream location FEN-D was clearly affected, while the upstream location (FEN-U) and the Long River (FEN-T) showed no or less impact, respectively. This suggests an input source between these points, like industry or domestic sewage disposal from Fengdu. The similar mutagenic potentials of WU-U and WU-D at Wushan suggest a drift of mutagenic compounds from upstream to downstream with no obvious influence from the Daning River (WU-T), with the source located upstream of WU-U. At the Hanfeng Lake, the cytotoxicity in sample HAN-C and the mutagenicity in sample HAN-D further corroborate a considerable input source between HAN-B and HAN-C (cf. Chapter III-2.3.1.).

Almost all mutagenic effects could be related to frameshift mutations after metabolic activation. Comparing this to the chemical results PAHs appear to be the obvious causative agents. Depending on the individual PAH they can either induce frameshift (TA98) or base pair substitution (TA100) (Madill et al. 1999), but generally require metabolic activation (WHO 2010). Chrysene for example is most probably the inducer for base pair substitution in sample YUN-D, because it constitutes 50\% (780 ng/g) of the PAH_{116} in the extract and reacts positively in tester strain TA100 with S9 (Madill et al. 1999).

Mutagenicity could also be detected in surface water of the Yangtze River at Chongqing by Shu et al. (2002) and Qiu et al. (2003) before the impoundment. Both stated that the Jialing River was stronger polluted than the Yangtze River. Further, direct- and indirect-acting frameshift inducers were found to be the causative agents in surface water of the Yangtze River at Wuhan section (Dong et al. 2010), Shanghai section (Shen et al. 2003a) and the Yangtze Estuary (Wu 2005), as well as in sediments of the German Rhine River (Kosmehl et al. 2004) and Danube River (Boettcher et al. 2010, Higley et al. 2012). Generally, surface waters appear to be dominated by direct- and indirect-acting frameshift mutagens worldwide (Ohe et al. 2004).
Table III-2.6. Mutagenic activity of sediment extracts determined in the Ames fluctuation assay with *Salmonella typhimurium* tester strain TA98 with exogenous enzymatic S9 supplement for metabolic activation of promutagenic compounds. Values given are No Observed Effect Concentrations (NOEC) in mg/mL.

<table>
<thead>
<tr>
<th>Chongqing</th>
<th>Fengdu</th>
<th>Yunyang</th>
<th>Wushan</th>
<th>Kaixian</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNG-U</td>
<td>CNG-T</td>
<td>CNG-D</td>
<td>FEN-U</td>
<td>FEN-T</td>
</tr>
<tr>
<td>50</td>
<td>25</td>
<td>200</td>
<td>200</td>
<td>25</td>
</tr>
</tbody>
</table>

*a* >400 mg/mL

2.3.3 *In vitro* EROD induction assay with RTL-W1 cells

The EROD induction assay with RTL-W1 cells was used to determine the Ah receptor mediated activity of sediment extracts. In campaign September 2011, the mean bio-TEQ EC25 ranged from 93 ± 13 pg/g (BJX-R) to 1,161 ± 326 pg/g (YUN-U) (Fig. III-2.3). The strongest inducing samples could be found at Yunyang (YUN-U), Chongqing (CNG-U), Kaixian (HF-L) and Wushan (WU-U), which also showed the only significant inductions compared to the reference site (BJX-R) (*p*<0.05). The pattern of EROD induction per site at the mainstream – with exception of Wushan - was: upstream > tributary > downstream. Inter-site comparison of the mean and total induction per site along the mainstream each showed the following order: Yunyang > Chongqing > Wushan > Fengdu (Fig. III-2.3).

In campaign May 2013 the mean bio-TEQ EC25 range was between 87 ± 13 pg/g (TGR-B) and 883 ± 100 pg/g (HAN-C), with the highest induction potentials at Kaixian (HAN-C; HAN-D), which also displayed the only significant induction compared to the reference site BJX-R (*p*<0.05) (Fig. III-2.3). At Chongqing, the change of induction potentials in the Yangtze River (YAN) increased with the flow direction, whereas they overall decreased in the Jialing River (JIA) – with a light drop in the middle section – and continued to decline in the TGR after the conversion zone. Inter-site comparison of the Yangtze (316 ± 99 pg/g) and Jialing River (309 ± 84 pg/g) showed comparable inductions, with lower activities in the TGR (164 ± 93 pg/g). At Kaixian, a clear increase could be observed between the upper (HAN-A; HAN-B; 90 ± 12 pg/g) and the lower (HAN-C; HAN-D; 824 ± 94 pg/g) section of the Hanfeng Lake (Fig. III-2.3). This indicates a pollution influx between those sites, as also observed by the chemical analysis, mutagenicity and cytotoxicity of the respective samples as described in Chapters III-2.2.1 and III-2.3.2. The potential origin was referred to the city of Kaixian on the south side and/or the Dong or Toudao River, which enter the lake on the north side.
Fig. III-2.3. Mean detected EROD inductions (Bio-TEQs) in comparison to calculated inductions of detected PAHs (Chem-TEQs) in sediment extracts from sampling campaigns September 2011 (A) and May 2013 (B). For each location the left bar relates to the Bio-TEQs on the left y-axis and the right bars to the Chem-TEQs on the right y-axis. Error bars represent standard deviations; n = 3; Percentages give differences between Bio-TEQs and Chem-TEQs (cf. Chapter III-2.3.6); dotted lines indicate different sites, double dotted lines separate sites along the Yangtze River mainstream and the TGR watershed; Asterisks mark significant differences between samples and reference (BJX-R); Data was statistically analyzed with ANOVA on ranks (multiple comparison) with Dunn’s post hoc test, * = p < 0.05.
In national and international comparison AhR-induced activity appeared to be in the medium to upper range. The obtained bio-TEQ EC25 values mainly exceeded those of crude extracts measured with RTL-W1 cells from the Yangtze Estuary (39-324 pg/g) (Liu et al. 2014), the German Elbe River Estuary (16 – 322 pg/g) (Otte et al. 2013) and European Danube River (20-123 pg/g) (Keiter et al. 2008), whereas compared to the Tietê River in Brazil (n.d. – 24,170 pg/g) (Suáres Rocha et al. 2010) and to the European Rhine River (2,387- 4,553 pg/g; 3,620 and 7,920 pg/g) (Schulze et al. 2014 and Heimann et al. 2011, respectively) activities ranged in the lower to medium range for most of the sediments investigated there.

A comparison of the EROD assay with the FET results revealed a highly significant correlation (September 2011: $r = 0.802$, $p<0.001$) (Fig. III-2.4), meaning samples with higher EROD induction also showed higher embryotoxic and teratogenic impacts, resembled by lower EC50 values. This hints to common inducers and possibly even to a contribution of the Ah receptor to the observed effects in the fish eggs (cf. Chapter III-2.4.5). The AhR plays a central role in the regulation of complex biological processes, like cell cycle progression, cellular differentiation, apoptosis and tumorigenesis (Marlowe & Puga 2005, Hahn et al. 2009, Ma et al. 2009). It is proposed that a hyper-activation of the AhR - by TCDD and other dioxin-like
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compounds - leads to a misregulation of target genes integrated in these basic processes, further resulting in the manifestation of toxic effects (Carney et al. 2004).

2.3.4 In vivo Fish Embryo Toxicity Test and Sediment Contact Assay with Danio rerio

The SCA and FET were performed to record the lethality and overall embryotoxic/teratogenic effects of bioavailable (SCA) and extracted (FET) compounds on embryos and larvae of Danio rerio after 96h. In the SCA all samples of campaign 2011 showed lethality and overall effects (lethal plus sublethal) on the fish only in range of the negative control (0-10%) (data not shown). The screening of the extracted samples of campaign of May 2013 in the highest concentration (100 mg/mL) showed no effects, thus neither SCA nor FET were conducted in detail.

In the FET for campaign 2011 lethal impacts could only be registered for the samples from Long River (FEN-T; LC50: 50 mg/mL; 95% CI: 46-54 mg/mL; \( r^2 = 0.95 \)) and Hanfeng Lake (HF-L; LC50: 30 mg/mL; 95% CI: 26-34 mg/mL; \( r^2 = 0.96 \)). For all other samples the LC50 was larger than the highest tested concentration of 100 mg/mL. In comparison, Wu et al. (2010) determined mortalities between 3 to 16 % for sediment extracts (concentration: 60 mg/mL) from the Yangtze Estuary after 96 h exposure, whereas in this study mean mortalities (± SD) ranged from <10% in most of the samples up to 80 ± 10% (concentration: 60 mg/mL) in the Long River (FEN-T) and 80 ± 17% (concentration: 50 mg/mL) in the Hanfeng Lake. Schiwy et al. (2014) determined LC50 values of 160 mg/mL and 355 mg/mL for sediment extracts from Altrip and Ehrenbreitstein, respectively, in the FET after 96 h exposure. Both are sampling sites at the German Rhine River and have been classified previously to be moderately and low contaminated. Whereas they could determine a LC50 value of 1 mg/mL for sediment extracts from the Vering Canal at Hamburg, Germany, known as to be highly burdened with old environmental load (Feiler et al. 2009, Höss et al. 2010).

The overall effects (lethal plus sublethal) of the sediment extracts in the FET ranged from EC50 16 mg/mL (HF-L; 95% CI: 14-18 mg/mL) to 121 mg/mL (BJX-R; 95% CI: 108-135 mg/mL). The goodness of fit was in all cases \( r^2 \geq 0.89 \). None of the samples had an overlapping 95% confidence interval with the reference site (BJX-R), thus all sites can be considered to display significant effects (Brandstätter 1999) (Fig. III-2.5). The most toxic samples could be found at Kaixian (HF-L; 16 mg/mL), Chongqing (CNG-U; 28 mg/mL) Yunyang (YUN-U; 30 mg/mL) and Fengdu (FEN-U; 33 mg/mL). The pattern of overall effects (lethal plus sublethal) at the mainstream – with exception of Wushan – was: upstream > tributary > downstream, analogous to the observations made with the EROD assay (Fig. III-2.3) (cf. Chapter III-2.4.4).
Fig. III-2.5. Overall effects on *Danio rerio* exposed for 96h to sediment extracts from sampling campaign September 2011 given as half maximal effective concentration (EC50). Overall effects are constituted by the sum of lethal endpoints and sublethal endpoints (cf. Chapter II-2.6.5). Symbols represent mean values and error bars 95% confidence intervals; n=3; dashed line indicates highest tested concentration 100 mg/mL; dotted lines indicate different sites, double dotted lines separate sites along the Yangtze River mainstream and the TGR watershed.

Overall can be stated that most samples did not induce significant mortality in the fish eggs after 96h. Aside the acute lethal effects numerous effects could be recorded that might result into severe consequences on the long run. The main set of effects were coagulation, yolk sac and pericardial edema, as well as disruptions of the cardiac function, manifested as diminished heartbeat and blood-circulation, or a total lack of both. A number of PAHs, PCBs and related dioxin-like compounds are known inducers of developmental disorders, among them the observed edema of the pericardium (Cantrell et al. 1998, Sundberg et al. 2005) and decreased blood circulation (Cantrell et al. 1998, Teraoka et al. 2010). Further, Incardona et al. (2004) described cardiac dysfunction, edema, spinal curvature and reduction in the size of the jaw and other craniofacial structures as part of a characteristic suite of abnormalities after exposure to complex PAH mixtures from petrogenic sources. They further highlighted secondary consequences of defects in the cardiac function on later stages of cardiac, renal, nervous and craniofacial morphology. Brette et al. (2014) demonstrated a cardiotoxic mechanism, by which
water accommodated fractions of PAHs containing crude oil had a serious impact on the regulation of cellular excitability via direct effects on ion channels, with consequences for life-threatening arrhythmias in fish and other vertebrates. The detrimental impacts of PAHs on teleost, avian, or mammalian systems could be shown in a number of studies (Barron et al. 2004).

Although the measured CYP1A activity in the EROD assay significantly correlated with the manifestation of effects in the FET in this study, and another CYP1 family could be associated with midbrain blood-flow after TCDD exposure (Kubota et al. 2011), the mode-of-action of dioxin-like compounds should not be regarded solely to the CYP1 family. Rather, the role of the AhR, with its multiple participation in several basic biological processes - aside its initiating role in CYP1 expression - should be emphasized. It has been shown that the AhR is constitutively active during development of the \textit{D. rerio} embryo and AhR repressors regulate its activity. A knockdown of certain repressors led to dioxin-like developmental phenotypes - presumably by hyperactivation of the AhR -, but had no effect on CYP1A expression, which suggests that not all dioxin-like phenotypes are dependent on CYP1A expression (Carney et al. 2004, Jenny et al. 2009).

2.3.5 Correlation between bioassays and chemical analysis

In order to identify potential correlations the detected chemical burden was compared to the effects in the bioassays.

Firstly, EC50 values obtained in the FET (FET\textsubscript{EC50}) - with a broad spectrum of possible organism-pollutant interactions – were compared to the results of the chemical analysis (cf. Chapter III-2.2.1). Among 54 organic compounds only 16 PAHs could be detected. The total content (PAH\textsubscript{16}) ranged between 165 and 1,653 ng/g for campaign 2011 and between 127 and 590 ng/g for campaign 2013 (cf. Chapter III-2.2.1). A significant correlation could be shown for the FET\textsubscript{EC50}-PAH\textsubscript{16} comparison (September 2011: \( r=0.552; p<0.05 \)) (Fig. III-2.6), meaning that the EC50 decreases – and toxicity increases – with increasing PAH\textsubscript{16} amount.

In addition, bio-TEQ values obtained in the EROD assay - with a mechanism specific mode of action - were compared only to the sum of PAHs with EROD induction capacity (PAH\textsubscript{EROD}). Those were benzo[k]fluoranthene, dibenzo[a,h]anthracene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, benzo[b]fluoranthene, chrysene, benzo[a]anthracene, as listed by decreasing potency (Bols et al. 1999). Here significant positive correlations could be demonstrated (September 2011: \( r=0.659, p<0.05 \); May 2013: \( r=0.802; p<0.001 \)) (Fig. III-2.7), after
exclusion of sample YUN-D, which appeared to reflect a special situation. Although YUN-D displays a comparably low EROD induction it contains one of the comparably highest amounts of PAH\textsubscript{EROD} (1,118 ng/g). This observation can be explained by the large abundance of chrysene (780 ng/g) in that sample, which has a comparably low specific induction potential. However, the observed correlations in the FET and EROD corroborate the suggestion that PAHs are among the responsible compounds that triggered the CYP1A induction and phenotypes of \textit{D. rerio}.

However, although a relationship could be shown between the chemical analysis and the EROD assay and the FET, the total concentrations of PAHs in this study that induce mutagenicity in the Ames assay did not correspond to the observed effects in the individual samples. Also, no obvious pattern in the intensity of mutagenic effects was discernible comparing the sampling locations, although the tributaries - with exception of CNG-T - in general showed the lowest mutagenic properties per sampling site (Table III-2.6).

Fig. III-2.6. Correlation of the results from the \textit{in vivo} Fish Embryo Toxicity Test (1/EC50) after exposure to sediment extracts from campaign September 2011 compared to the total content of PAHs (PAH\textsubscript{\leq 6}) in the respective extracts, given as linear regression. Dashed lines limit the 95\% confidence interval of the fitted data. As a decreasing EC50 indicates increasing toxicity, it has to be noted that the original EC50 values have been transformed to 1/EC50 in order to achieve a positive correlation for better comprehensibility – a higher PAH concentration equals stronger effects in the FET.
Fig. III-2.7. Correlations of the results from the *in vitro* EROD induction assay (mean BioTEQ EC25) after exposure to sediment extracts from (A) campaign September 2011, as well as (B) campaign May 2013, compared to the total content of PAHs that are active in the EROD assay with RTL-W1 cells (PAH_{EROD}) (Bols et al. 1999) in the respective extracts, given as linear regression. Dashed lines limit the 95% confidence interval of the fitted data. As a decreasing EC50 indicates increasing toxicity, it has to be noted that the original EC50 values have been transformed to 1/EC50 in order to achieve a positive correlation for better comprehensibility – a higher PAH concentration equals stronger effects in the FET.
2.3.6 Responsible compounds in the in vitro/in vivo assays

In order to identify the individual contribution of the analyzed compounds to the observed effects so called toxicity equivalents (TEQs) can be calculated in relation to a strong inducer.

Although all 16 priority PAHs (Table II-2) may induce adverse effects, not all of them are mutagenic. Particularly those with more than four rings possess adverse mutagenic properties, but often also require metabolic activation (Penning et al. 1999). Out of the selected 16 priority PAHs eight possess mutagenic activity in the Ames assay (Table III-2.1; Table III-2.2). The proportion of those in the sediment samples from September 2011 range from 43% (CNG-U) to 79% (YUN-D), with a mean of 55 ± 9%. The proportion in the samples from May 2013 range from 39% (JIA-A; JIA-B) to 59% (YAN-A), with a mean of 48 ± 8% at Chongqing, and 36% (HAN-A; HAN-B) to 49% (HAN-C), with a mean of 42 ± 7% at Kaixian.

As each PAH has a specific mutagenic activity, the individual inductions can be calculated in relation to a strong mutagen, e.g., benzo[a]pyrene. However, the application of benzo[a]pyrene toxicity equivalency factors (BEP or BaP-TEQ), according to (Madill et al. 1999), also resulted in no clear trend between the activity of detected compounds and the triggered effects. Therefore, it is suggested that non-target compounds may play a significant role in the induction of mutagenicity (Brack et al. 2005). In other studies about 10-20% of the total mutagenicity was attributed to PAHs and the rest to non-target compounds (Chen & White 2004, Aouadene et al. 2008). The cytotoxicity in several samples, with comparably low PAH_{2-16} content (JIA-A, JIA-B), and the direct mutagenicity (-S9) in sample CNG-D, corroborates the assumption of toxic non-target compounds with potential mutagenic properties.

As already highlighted in Chapter III-2.3.5 only seven PAHs induce EROD activity in RTL-W1 cells. The proportion of those in the sediment samples from September 2011 range from 33% (CNG-U) to 72% (YUN-D), with a mean of 43 ± 9%. The proportion in the samples from May 2013 range from 24% (TGR-B) to 42% (YAN-A), with a mean of 34 ± 7% at Chongqing, and 23% (HAN-A; HAN-B) to 42% (HAN-C), with a mean of 32 ± 11% at Kaixian.

To further identify the potential contribution of the detected PAHs to the registered EROD induction, their individual inductions were calculated as chem-TEQs in relation to the strong inducing dioxin TCDD. The chem-TEQs were compared to the bioassay derived bio-TEQ values from the EROD assay (Fig. III-2.3). The detected PAHs in the sediments showed a
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contribution of 6-25% to the overall AhR-mediated activity in campaign September 2011 and of 1-23% in campaign May 2013 - assuming only additive effects.

While Otte et al. (2013) could refer the majority of activity to priority PAHs in most of the samples from the Elbe Estuary, only a minority of the activity could be explained by PAHs in sediment studies about the Yangtze Estuary (max. 10%), the Tietê River (max. 7%) and most of the samples of the Danube River (max. 17%) (Keiter et al. 2008, Suares Rocha et al. 2010, Liu et al. 2014). A part of the missing activity in those studies could be explained by other persistent pollutants - like PCBs, polychlorinated dibenzodioxins (PCDDs) and –furans (PCDFs) – as isolated by multilayer fractionation (Keiter et al. 2008, Otte et al. 2013, Liu et al. 2014). However, PCBs as well as several pesticides, like atrazine, and several other compounds can be excluded as causative agents as they could not be detected in the sediment extracts of this study (cf. Chapter III-2.2.1).

Therefore, the discrepancies between bio-TEQs and chem-TEQs can be referred most likely to differences in specific induction potentials and undetected non-target compounds (cf. Chapter III-2.5). Furthermore, additive, synergistic, antagonistic and masking interactions of detected and undetected pollutants impede explanations.

In any case, a comparison of the high induction of effects in the FET with no particular effects in the SCA suggests only a low bioavailability of the particle-bound pollutants. This further underlines the contribution of rather lipophilic substances to the observed effects.
2.4 Triad C: *In situ* biomarkers

2.4.1 Micronucleus assay

In the erythrocytes of *P. vachellii* sampled along the Yangtze River mainstream in May 2012 no significant differences (*p* > 0.05) in the micronucleus frequency could be detected at Chongqing (CNG 2012: 0.175 ± 0.121%; IF 0.9; *n* = 10), Fengdu (FEN 2012: 0.150 ± 0.129%; IF 0.8; *n* = 10), Yunyang (YUN 2012: 0.150 ± 0.129%; IF 0.8; *n* = 10) and Wushan (WU 2012: 0.175 ± 0.169%; IF 0.9; *n* = 10) compared to the reference site Baijiaxi River (BJX 2012: 0.200 ± 0.158%; *n* = 10). However, the samples taken from the Hanfeng Lake in 2012 displayed a significant effect (HF 2012: 0.400 ± 0.175%; IF 2.0; *n* = 10; *p* < 0.05) compared to the Baijiaxi River. Furthermore, the erythrocytes sampled in Chongqing (CNG 2013: 2.250 ± 2.073%; IF 11.3; *n* = 20; *p* < 0.001) and Hanfeng Lake (HF 2013: 1.450 ± 2.040%; IF 7.3; *n* = 20; *p* < 0.01) in May 2013 showed even stronger effects. Comparing the change between the years at Hanfeng Lake (HF 2012; HF 2013; *p* > 0.05) no significant difference could be measured, whereas at Chongqing (CNG 2012; CNG 2013; *p* < 0.001) the status changed significantly (Fig. III-2.8).

**Fig. III-2.8.** Micronucleus frequency in erythrocytes of *Pelteobagrus vachellii* from sampling campaign May 2012 and May 2013. Symbols represent individual animals, bars the mean value, and error bars the standard deviation; white circles = May 2012; black circles = May 2013; dotted lines indicate different sites, double dotted lines separate sites along the Yangtze River mainstream and the TGR watershed; 2012 samples *n* = 10; 2013 samples *n* = 20; Asterisks mark significant differences between samples and reference (BJX 2012); Data was statistically analyzed with Mann-Whitney Rank Sum Test, * = *p* < 0.05, ** = *p* < 0.01, *** = *p* < 0.001, a = significant difference to reference site, b = significant difference to same site in May 2012.
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The biological impairments may be attributed to PAHs, as detected at Chongqing and Kaixian in May 2013 (Table III-2.2). Genotoxic properties of PAHs could be demonstrated before (White 2002). Further, environmental sediments contaminated with PAHs have shown genotoxic effects in catfish (Di Giulio et al. 1993) and turbot (Kilemade et al. 2004), barbell (Boettcher et al. 2010), tilapia (Rocha et al. 2009), as well as resuspended PAH-spiked sediments in trout (Brinkmann et al. 2013, Hudjetz et al. 2014). The harbor sediments that caused DNA damage in turbot blood cells contained PAH concentrations of about 1,000 ng/g after 7 days of exposure, and the reference site – containing about 500 ng/g PAH - also induced significant effects after 14 days. Likewise, other organs – liver, epidermis, spleen and gill – were also affected significantly (Kilemade et al. 2004). Thus, although PAH_{16} concentrations were at 302 ± 87 ng/g at Chongqing and 332 ± 240 ng/g at Kaixian, the exposure time has to be taken into account, as the formation of micronuclei is dependent on contamination level and exposure time (Das & Nanda 1986). PAHs generally persist in the environment with half-lives from days to years – depending on the individual structure and processes like photo- and biodegradation (Shuttleworth & Cerniglia 1995, Kanaly & Harayama 2000, Fasnacht & Blough 2002) -, and can accumulate in organisms without the right metabolic detoxification systems due to their lipophilicity, and consequently biomagnify through trophic transfer (Coates et al. 1997, Kanaly & Harayama 2000). However, they are easily biodegradable in organisms with the right metabolic mechanisms (cf. Chapter III-3) (Van der Oost et al. 2003, Möller et al. 2014), unfolding their toxic potentials through biotransformation to reactive intermediates (WHO 2010).

Table III-2.7. Parameters for PAH metabolites in bile of *Pelteobagrus vachelli* from sampling campaign May 2012. Parameters were concentrations of 1-hydroxypyrrene (1-OHPyr) given as ng/mL, absorption of bile at 380 nm in dimensionless absorption units (A380) given as 1/mL, the concentration of 1-OHPyr normalized by A380 given as ng/A380, and the ratio of 1-OHPyr to 1-hydroxyphenanthrene (1-OHPhe) in dimensionless units; Values are stated as means with standard deviation of pooled samples (n); n = 3, with exception of WU 2012 (n = 2).

<table>
<thead>
<tr>
<th>Sites</th>
<th>1-OHPyr [ng/mL]</th>
<th>A380 [1/mL]</th>
<th>1-OHPyr per A380 [ng/A380]</th>
<th>1-OHPyr/1-OHPhe ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNG 2012</td>
<td>1120 ± 296</td>
<td>32 ± 21</td>
<td>47 ± 25</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>FEN 2012</td>
<td>1211 ± 460</td>
<td>61 ± 6</td>
<td>19 ± 6</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>YUN 2012</td>
<td>211 ± 88</td>
<td>10 ± 3</td>
<td>20 ± 3</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>WU 2012</td>
<td>2428 ± 871</td>
<td>5 ± 0</td>
<td>499 ± 146</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>HF 2012</td>
<td>1562 ± 212</td>
<td>43 ± 8</td>
<td>37 ± 6</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>BJX 2012</td>
<td>328 ± 201</td>
<td>22 ± 8</td>
<td>22 ± 22</td>
<td>7 ± 2</td>
</tr>
</tbody>
</table>

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2.4.2 PAH metabolites in bile of *Pelteobagrus vachelli*

In order to determine if PAHs could be detected in fish of the species *P. vachelli*, exemplarily for other species, the indicator metabolite 1-OHPyr – which contributes up to 76% to the sum of biliary PAH metabolites (Ruddock et al. 2003) - was analyzed in bile of the fish collected in campaign 2012. The presence of PAHs in the fish from campaign 2012 could be verified. The mean contents of 1-OHPyr ranged from 211 ± 88 ng/mL at Yunyang (YUN 2012) to 2,428 ± 871 ng/mL at Wushan (WU 2012) *(Table III-2.7).* A repeated determination of 1-OHPyr in samples from campaign 2013 was not possible due to very low available amounts of bile in the gall bladder.

Based on the normalized 1-OHPyr contents and the 1-OHPyr/1-OHPhe ratio *(Table III-2.7)* fish from Wushan clearly displayed the strongest contamination. This can either be referred to a higher pollution at Wushan, as Blahova et al. (2014) determined a statistically positive correlation between biliary 1-OHPyr and the total PAH content in the water, or to an extreme situation, like a contaminated food source that was taken up by the fish not long before they were caught. Most individuals from Wushan had an amply filled stomach and displayed the lowest absorption of bile at 380 nm *(Table III-2.7)*, meaning that bile was secreted for digestion of the food and freshly produced. Hence, less concentrated bile was measured. Overall, in comparison fish from the sites Baijiaxi and Yunyang displayed the lowest contamination levels *(Table III-2.7).*

The results of the present study were compared to concentrations of PAH metabolites in fish caught in freshwater and coastal habitats from other countries. The mean PAH metabolite concentrations in eel from European countries (range of means: 1-OHPyr: 56-3,200 ng/mL; 1-OHPhe: 70-495 ng/mL) are in the same order of magnitude than those found in *P. vachelli* obtained in this study (means: 1-OHPyr: 1,087 ng/mL; 1-OHPhe: 136 ng/mL) (Ruddock et al. 2003, Nagel et al. 2012, Kammann et al. 2014, Szlinder-Richert et al. 2014).

To evaluate the measured PAH metabolite concentrations with respect to a possible harm of PAH contamination for the organisms, the results were compared to internationally agreed threshold values for fish. 12 of 17 investigated pool samples exceeded the threshold value for 1-OHPyr for cod (Garbus morhua) of 438 ng/mL bile, and 9 of 17 pool samples exceeded the threshold value for turbot (Scopthalmus maximus) of 909 ng/mL (ICES 2012). Even if this threshold has been calculated for another fish species, this comparison indicates that the PAH contamination in fish from China is of significance and may rise concern for the fish health in
some cases. To the author’s knowledge no such thresholds exist for freshwater fish. However, this comparison gives only an impression on single contaminants and might not reflect the overall situation of organic pollution. Although, a good relationship between PAH content of resuspended sediment, uptake and biliary PAH metabolites could be demonstrated in rainbow trout by Brinkmann et al. (2013). Further, the cited threshold values provide only a rough guidance because they have been calculated for marine fish. Beyond, due to the low number of available replicates these results should only be considered as indications.

2.4.3 Nutrition status and condition of *Pelteobagrus vachellii*

The determined bile color (A380) also allows an interpretation of the short term nutrition status of the fish caught in campaign 2012, as an increase in concentration of bile pigments can be expected during starvation periods of fish (Richardson et al. 2004, Kammann et al. 2014). Fish from Wushan (WU 2012) and Yunyang (YUN 2012) seemed to have the best status, while fish from Kaixian (HF 2012) and Fengdu (FEN 2012) appeared to have not eaten for the longest time in comparison (Table II-1). Significant differences could be found between WU 2012 with FEN 2012, HF 2012 and CNG 2012, as well as between YUN 2012 with FEN 2012 and HF 2012, in addition to FEN 2012 compared to BJX 2012 ($p<0.05$).

However, the long-term condition of the fish can be judged based on the Fulton condition factor (k-value; here: total length) (Table II-1). It ranged from $0.75 \pm 0.08$ (FEN 2012) to $1.01 \pm 0.08$ (CNG 2012) in 2012 and from $0.83 \pm 0.10$ (HF 2013) to $0.96 \pm 0.12$ (CNG 2013) in 2013. Significant differences could be determined between FEN 2012 with CNG 2012, HF 2012 and BJX 2012, further between CNG 2012 with YUN 2012 for samples from campaign 2012. For samples from campaign 2013 significant differences could be registered between CNG 2013 and HF 2013, as well as between HF 2013 and the reference BJX 2012 ($p<0.05$). Both campaigns showed almost matching k-values, which underlines the consistency of this parameter over the years.

Apparently fish from Chongqing seemed to be in the comparably best fed condition, whereas fish from Fengdu exhibited the comparably less fed status. The better nutrition status at Chongqing may be regarded to a higher supply of food the catfish can prey on, due to a higher supply of organic matter with origin in elevated levels of wastewater in the highly urbanized area.
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Table III-2.8. Fulton condition factors (k-values) of *Pelteobagrus vachellii* and other catfish species caught from fish stocks or as wildlife. Condition factors are given as mean values with standard deviation; sexes are given as female (f), male (m) or combined (c; male plus female); Condition factors were calculated with total length (tot) or standard length (std).

<table>
<thead>
<tr>
<th>Country</th>
<th>Site</th>
<th>Month</th>
<th>Species (family)</th>
<th>Sex</th>
<th>k-value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>River, lake</td>
<td>May (2012)</td>
<td><em>Pelteobagrus vachellii</em> (Bagridae)</td>
<td>C</td>
<td>0.90 ± 0.13 (tot) 1.40 ± 0.18 (std)</td>
<td>This study</td>
</tr>
<tr>
<td>China</td>
<td>River, lake</td>
<td>May (2013)</td>
<td><em>Pelteobagrus vachellii</em> (Bagridae)</td>
<td>C</td>
<td>0.90 ± 0.13 (tot) 1.40 ± 0.25 (std)</td>
<td>This study</td>
</tr>
<tr>
<td>China</td>
<td>Stock</td>
<td>October</td>
<td><em>Pelteobagrus vachellii</em> (Bagridae)</td>
<td>C</td>
<td>0.92±0.06 up to 1.00±0.10 (tot)</td>
<td>(Zeng et al. 2010)</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>River</td>
<td>Year</td>
<td><em>Mystus vitatus</em> (Bagridae)</td>
<td>F</td>
<td>2.32 (std)</td>
<td>(Hossain et al. 2006)</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>River</td>
<td>Year</td>
<td><em>Mystus vitatus</em> (Bagridae)</td>
<td>M</td>
<td>2.20 (std)</td>
<td>(Hossain et al. 2006)</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>River</td>
<td>May</td>
<td><em>Gagata cenia</em> (Sisoridae)</td>
<td>C</td>
<td>1.88 ± 0.22 (std)</td>
<td>(Chaki et al. 2013)</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Lagoon</td>
<td>Year</td>
<td><em>Chrysonchthys nigrodigitatus</em> (Bagridae)</td>
<td>C</td>
<td>0.81 ± 0.20 (tot)</td>
<td>(Fafioye &amp; Oluajo 2005)</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Lagoon</td>
<td>Year</td>
<td><em>Chrysonchthys walkeri</em> (Bagridae)</td>
<td>C</td>
<td>1.15 ± 0.96 (tot)</td>
<td>(Fafioye &amp; Oluajo 2005)</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Lagoon</td>
<td>Year</td>
<td><em>Clarias gariepinus</em> (Clariidae)</td>
<td>C</td>
<td>0.79 ± 0.15 (tot)</td>
<td>(Fafioye &amp; Oluajo 2005)</td>
</tr>
</tbody>
</table>

To the author’s knowledge no k-values are available for wildlife *P. vachellii* from other studies so far. Zeng et al. (2010) computed condition factors (total length) between 0.92 ± 0.06 and 1.00 ± 0.10 for stocked juvenile *P. vachellii* under various stocking density conditions. The weight varied between 26.7 ± 7.9 g and 41.6 ± 12.8 g, the total length was in a range of 137.8 ± 13.1 mm to 159.5 ± 15.9 mm and the age was about five months (May to October). The common length of *P. vachellii* is given as 210 mm (Nichols 1943), and the maximum body length as 300 mm (Chan & Ho 2011). Based on this, the fish caught in this study can be classified to be in a juvenile to adult state, as average size and weight slightly exceeded the values by Zeng et al. (2010) (Table II-1). It is further supported by the observation that the reproductive organs - testes and ovaries - in most of the fish of this study showed only incomplete maturation. A reduced abundance of larger fishes could be observed during the sampling, which stands in accordance with Chen et al. (2009), who described a shift in the age structure of the catch from the upper Yangtze River - including the TGR region – from proportional decrease of older fish and an increase of juveniles and younger adult fish. Although
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k-values consider allometry of growth, and thus differ with age, a comparison with the k-values obtained in this study (k = 0.90 ± 0.13; total length) hints at a rather less good condition of the fish from the TGR (Table II-1).

For means of further interpretation, k-values were compared to other catfish species caught in rivers and lagoons. Overall, k-values (standard length) obtained in this study were clearly lower compared to those of *M. vittatus* and *G. cenia*, as well as the k-value (total length) of *C. walkerii*. Comparable k-values (total length) could be shown for *C. nigrodigitatus* and *C. gariepinus* (Table III-2.8). Variations between species, gender, age and season have to be considered. Hussain et al. (2006) could register a significant difference (p<0.05) between sampled males and females, and a change of the condition over the course of the year with the lowest in April and the highest in August, but could not determine any significant differences between the months (p>0.05). Overall, these results support the assumption that the fish from the TGR are in a rather poor condition.

2.4.4 Induction of hepatic EROD and GST activities in *Pelteobagrus vachelli*

As bile analysis showed a presence of PAHs in the fish, activities of Ah receptor mediated phase I (EROD) and phase II (GST) biotransformation enzymes were tested in excised liver samples from *P. vachelli*. The mean EROD activities for samples from campaign 2012 (n=10) and 2013 (n=20) ranged – with exception of Chongqing - from 2 ± 2 pmol/mg/min (HF 2013; IF 0.6) to 4 ± 3 pmol/mg/min (HF 2012; IF 1.2) and showed no significant differences (p>0.05) compared to the reference site Baijiaxi River (BJX 2012; 4 ± 3 pmol/mg/min). However, samples from Chongqing stood out significantly both from campaign 2012 (CNG 2012; 13 ± 9 pmol/mg/min; IF 3.6; p<0.05) and campaign 2013 (CNG 2013; 19 ± 10 pmol/mg/min; IF 5.1; p<0.001) in comparison to the reference site. The difference between the years comparing the samples from Chongqing are not significant (p>0.05) (Fig. III-2.9).

Further, the mean GST activities varied between 133 ± 56 nmol/mg/min at Fengdu (FEN 2012) to 168 ± 32 nmol/mg/min at Wushan (WU 2012) from samples of campaign 2012 (n=10), and between 143 ± 48 nmol/mg/min at Chongqing (CNG 2013) to 173 ± 49 nmol/mg/min at Kaixian (HF 2013) from samples of campaign 2013 (n=20). However, none of the samples exhibited a significant difference compared to the reference site (p>0.05) (Fig. III-2.9).
Fig. III-2.9. Hepatic EROD (A) and GST (B) activities in excised livers from *Pelteobagrus vachellii* determined for sampling campaign May 2012 and May 2013. Symbols represent individual animals, bars the mean value, and error bars the standard deviation; white circles = May 2012; black circles = May 2013; dotted lines indicate different sites, double dotted lines separate sites along the Yangtze River mainstream and the TGR watershed; 2012 samples n = 10; 2013 samples n =20; Asterisks mark significant differences between samples and reference (BJX 2012); Data was statistically analyzed with Mann-Whitney Rank Sum Test, * = p<0.05, *** = p<0.001, a = significant difference to reference site; significant difference to same site in May 2012 were calculated but no significances could be determined.
The results claim no significant correlation between the biliary 1-OHPyr content and the hepatic EROD or GST activities, as well as between the EROD and GST activities themselves (campaign 2012: p>0.05). A reasonable explanation for a lack of correlation despite present PAH metabolites could be that pyrene – the precursor to 1-OHPyr – is not regarded as an EROD inducer as described by Bols et al. (1999) in RTL-W1 cells. In contrast, Hudjetz et al. (2014) could show a similar but less pronounced gradient for EROD induction compared to 1-OHPyr increase in bile of rainbow trout exposed to sediment spiked with 16 PAHs in various concentrations in a laboratory experiment. 1-OHPyr is therefore considered as an indicator for PAH presence, but not as a guarantor for AhR inducers.

However, the biliary content of hydroxylated PAH metabolites at all sites demonstrate that PAH parent compounds must have been present and undergone metabolism. Brinkmann et al. (2013) proposed a biomarker cascade for rainbow trout exposed to resuspended PAH-spiked sediment, in which the peak in EROD induction preceded the peak of biliary PAH metabolites 1-hydroxypyrene, 1-hydroxyphenanthrene and 3-hydroxybenzo[a]pyrene. This is consequential, because CYP1A belong to the phase I enzymes that catalyze the reaction from the parent compounds to the metabolites, which then accumulate in the bile. Thus, a temporal variable may also be included into the situation: A peak exposure might have passed, followed by EROD induction in the liver. While an elevated enzymatic activity could not be observed anymore at the time point of sampling, the products still could be detected in the bile.

A loss of EROD activities as a result from inconsistent cooling can be excluded due to the registered GST activities obtained from the same S9-mix. Furthermore, although it has been shown that several PAHs induce EROD and GST activity in liver tissues, basal activities could be determined for both systems under absence of PAHs. Moreover, GST induction appeared to be very modest compared to CYP1 expression (Pushparajah et al. 2008a,b). The background activity of the enzymes may as well be regarded to a chronic exposure to low levels of xenobiotics. However, it can be concluded that the CYP1A system was not activated to a significantly higher degree at most of the sites with regard to the Pengxi River Nature Reserve as reference site (BJX-R) at the time point of sampling.

In contrast to that, the highly industrialized area of Chongqing city triggered a significant up-regulation of AhR-mediated CYP1 enzymes in fish from campaign 2012 and 2013. This elevated level of activity in phase I of the hepatic detoxification system hints at environmental stressors at this site, including AhR agonists. Moreover, as a consequence the catalytic activity of CYP1A enzymes can lead to a bioactivation of present PAHs to reactive intermediates, which
in their turn can induce mutagenicity (Penning et al. 1999). Significant genotoxic impacts could be observed on erythrocytes of *P. vachellii* from Chongqing (campaign 2013), as well as from Hanfeng (campaign 2012 and 2013) determined with the micronucleus assay, although samples from Chongqing from campaign 2012 turned out to be not affected significantly (cf. Chapter III-2.4.1).

Overall, whereas the *in vitro* EROD induction assay with RTL-W1 cells could be linked to the PAH content in the sediment extracts (cf. Chapter III-2.4.6), *in situ* hepatic EROD and GST activities could not be directly correlated to the results from the bioassay and chemical analysis obtained in parallel in campaign 2013. At the site Chongqing city, the *in vitro* EROD induction by sediment extracts ranged between 0.9 (TGR-B) to 4.2 fold (YAN-C) - with a mean of 3.0 fold –, and at Kaixian from 1.0 (HAN-B) to 9.5 fold (HAN-C) – with a mean of 4.9 fold - when set in relation to the reference (BJX-R). The hepatic EROD activity of the benthic *P. vachellii*, which should be in close contact with the sediment, showed a 5.1 fold induction at Chongqing (CNG 2013) and 0.6 fold at Kaixian (HF 2013) compared to the reference (BJX 2012). Moreover, integrating the temporal and spatial variations, by creating a mean of all *in vitro* EROD inductions (sediment), as well as a mean of all *in situ* hepatic EROD inductions (fish) of samples from the site Chongqing (campaigns 2011, 2012 and 2013), showed a 4.1 fold *in vitro* EROD and 4.6 fold hepatic EROD induction relative to the respective reference. At Kaixian, the same approach resulted in a 6.2 fold *in vitro* EROD and 0.9 fold hepatic EROD induction. So, a relationship may apply for the situation at Chongqing, but not for Kaixian (for overview cf. Chapter III-2.7). The discrepancy at Kaixian may be regarded to the strong spatial variation of pollution between the upper and lower part of the Hanfeng Lake as registered in 2013 (cf. Chapter III-2.2.1). As the catch was obtained from the fishermen at the middle of the lake, the main origin of the fish may as well be the less polluted upper section, where the *in vitro* EROD induction was found to be 1.0 fold (HAN-A, HAN-B) relative to the reference, which is similar to the EROD induction found in the fish. Additional monitoring would be required to verify this observation.

In general, increase and decrease of EROD and GST activities are transient (Pesonen et al. 1987, Brinkmann et al. 2013) and the complex situation including bioavailability of the compounds, exposition and uptake pathways of the fish in combination with metabolization and secretion processes aggravate a direct correlation.
2.4.5 Histopathological evaluation of *Pelleobagrus vachellii*

Liver and kidney play a crucial role in the biotransformation and secretion of xenobiotics (Pesonen et al. 1987), hence they are frequently exposed to these compounds under contamination conditions. In their turn xenobiotics may leave their mark on the organs, which are therefore well suited for histopathological investigations, as applied for fish from campaign 2012.

In the kidney, infiltration with macrophages and giant cells (Chongqing, Fengdu, Wushan), multifocal accumulation of macrophages (Yunyang, Wushan), degeneration of melanomacrophage aggregates (Chongqing), as well as deposition of foreign material - probably degenerated parasitic structures - (Chongqing) could be observed (Fig. III-2.10). However, integrated over the whole catch of fish none of the mentioned criteria was significant in its grade of manifestation compared to the reference site (BJX 2012; \( p > 0.05 \)). Still, these criteria can be related to inflammatory reactions as a response to foreign material in the tissues, e.g. parasites. At Chongqing, 30 %, as well as Fengdu, 10 %, and Wushan, 10 % of the catch, were afflicted by the trematode *Isoparochis hypselobagri* (Billet, 1898) (Fig. III-2.11), which infested the swim bladder next to the kidney, and the digestive tract. The abundance of parasites at Chongqing could be verified in campaign 2013 (CNG 2013; 20%).
**Fig. III-2.10.** Plate of histological alterations of liver and kidney with surrounding tissue from *Pelteobagrus vachelli* from sampling campaign May 2012. A. Chongqing, degenerated macrophage center (star), intermixed multiple structures with a cyst-like wall and a homogenous degenerated center (open and closed arrowheads), interpreted as foreign material, probably degenerated parasitic structures, macrophages, lymphocytes and plasma cells infiltrating the surrounding fibrous tissue (arrow), H&E, bar=50μm; B. Fengdu, multiple structures resembling parasitic eggs with a yellowish wall and an eosinophilic homogenous center (open arrowheads), surrounded by a moderate amount of macrophages (closed arrowhead), embedded in a macrophage center (star), H&E, bar=25μm; C. Wushan, liver, unaffected liver structure with bile duct (arrow), H&E, bar=25μm; D. Baijiaxi, liver, bile duct proliferation with degeneration of bile duct epithelial cells expressed by karyopyknosis (arrowheads), surrounded by fibroblasts (fibrosis) and lymphocytes, plasma cells and fewer macrophages (arrows), H&E, bar=25μm; E. Baijiaxi, kidney, unaffected kidney structure, H&E, bar=25μm; F. Wushan, kidney, infiltration with mainly macrophages in the interstitial tissue (open stars), surrounding a vessel, multiple inflammatory cells in the vessel lumen, H&E, bar=25μm.
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The appearance of the trematodes also closely correlated with the occurrence of infiltration with macrophages, degeneration of melanomacrophage aggregates and deposition of foreign material in the livers of fish from Chongqing. Furthermore, lymphoid tissue around biliary tracts (Fengdu, Yunyang, Hanfeng Lake, Baijiaxi River) and bile duct proliferation (Yunyang, Hanfeng Lake, Baijiaxi River), as well as degeneration of hepatocytes and bile duct epithelial cells, expressed by karyopyknosis (Baijiaxi River) could be observed in the liver (Fig. III-2.10). These primarily account for more chronic degenerative and inflammatory reactions. Here also, integrated over the whole catch of fish, none of the mentioned criteria was significant in its grade of manifestation compared to the reference site (BJX 2012; p>0.05).

Although not significant, the observed effects can indicate negative environmental impacts. The recorded macrophage aggregates are reported to increase in size and frequency in conditions of environmental stress and have been proposed as biomarkers for water quality for pollution and deoxygenation as reviewed by Agius and Roberts (2003). Camargo and Martinez (2007) as well as Belicheva and Sharova (2011) ranked macrophage aggregates among other structural changes to responses to pollution obtained from in situ studies at a Brazilian urban stream and a Russian reservoir under anthropogenic influence.

![](image1)

Fig. III-2.11. Monitoring fish species *Pelteobagrus vachellii* (left) afflicted with trematode *Isoparochis hypselobagri* (right) displaying the typical symptom of intestines covered in dark mucus. Bar = 0.5 cm.
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Further, pollution can suppress the immune system of fish, thus increasing the chance for parasites to enter and settle in the host, if the host is more susceptible to the pollution than the parasite (Khan & Thulin 1991, MacKenzie et al. 1995). PAHs and phenols have been shown to suppress non-specific cytotoxic cells in teleosts, which are relevant in the immunologic defense from xenogenic targets, like certain fish parasites (Graves et al. 1985, Faisal et al. 1991, Seeley & Weeks-Perkins 1997, Taysse et al. 1998, Reynaud & Deschaux 2006). Beyond, Möller et al. (2014) demonstrated that PAHs are distributed into the immune organs head kidney and spleen in fish, and that those possess the capability to metabolize PAHs to potentially immunotoxic metabolites (cf. Chapter III-3), which in their turn may diminish immunological responses (Khan & Thulin 1991). Further, an increased pollution of the water with organic material can cause an increased reproduction of macroinvertebrates that can serve as intermediate hosts. According to Manna and Das (2003) the freshwater snail Indoplanorbis exustus (Deshayes, 1834) is a first intermediate host of I. hypselobagri in India. An increased amount of intermediate hosts allow a propagated reproduction of the parasites, consequently causing increased infection rates in the fish. The comparably higher frequency of parasites found at the strongly industrialized site of Chongqing, hence may be used as an indicator for environmental degradation (Khan & Thulin 1991).

The registered karyopyknosis at the Baijiaxi River, which indicated apoptosis of hepatic cells, further suggests effects of pollution also on the fish from the reference site. Hepatic lesions could be significantly correlated in other studies to total PAHs in bottom sediment, as well as metabolite concentrations in marine fish (Krahn et al. 1986, Landahl et al. 1990). However, the exposure must have been some time prior to the sampling, as all other analyses suggest only a minor influence of the site on the fish and the organ lesions show a more chronic character. One reason could be a local discharge, another an influence of the reservoirs backwater on the Baijiaxi River from downstream, due to the rise of the water level in the winter months prior to the sampling campaign in May 2012. Holbach et al. (2013, 2014) recorded a reversion of the flow direction at the Daning and Xiangxi River – both main TGR tributaries -, forced by the reservoirs increasing water level. The Pengxi River, in which the Baijiaxi River discharges into further downstream of the reference site BJX-R, connects the city of Kaixian – as well as several industrial sites along its shore - with the TGR and exhibits frequent shipping activities (Fig. II-4). As transitions in water level can be even noticed in the Pengxi-Baijiaxi River system, as consequence of the reservoirs water level fluctuation, it is suggested that a potential contamination in Pengxi River may be pushed upstream into the Baijiaxi River and affected the fish.
2.5 Non-target compounds

The identification of PAHs in the sediments was not sufficient to explain the entire responses measured in the *in vitro/in vivo* assays (cf. Chapter III-2.3.6). Thus, the involvement of non-target compounds has been suggested, which may also contribute to the effects assessed *in situ* with *Pelteobagrus vachellii*.

Possible non-target compounds could be sediment affine derivatives of the here ubiquitously detected PAHs. For example, Nitro-PAHs have also been demonstrated to possess frameshift and basepair substitution mutagenic potentials (McCoy et al. 1981, Xu et al. 1982, Eisentraeger et al. 2008), to induce CYP1A1 activity (Jung et al. 2001) and genotoxic effects in fish (Shailaja et al. 2006a). Higley et al. (2012) found primarily PAHs, nitro-PAHs, dinitro-PAHs, sterols and naphthoic acids to be associated with ecotoxicological impacts of sediment extracts from the Danube River, whereas PCBs and several other organic pollutants could be excluded.

Nitro-PAHs emerge from the combustion of fossil fuels at industrial facilities, coal-fired power plants, urban emission and vehicle exhaust, which were found to be considerable sources along the TGR particularly in the upper section (cf. Chapter III-2.2.5) (Harris et al. 1984, Talaska et al. 1996). They can also form in the environment from the reaction of certain PAHs with the highly reactive nitrite, which may originate from biological processes and nitrogenous waste like fertilizers, or by metabolic processes in fish under presence of both precursors. Shailaja et al. (2006a,b) demonstrated the formation of Nitro-PAHs and elevated EROD induction under presence of phenanthrene and nitrite. The simultaneously exposure to nitrite and the actually noncarcinogenic PAH phenanthrene even led to a potentiation in genotoxicity (Shailaja et al. 2006a), corroborating the importance of pollutant interactions in the expression of PAH toxicity (Chaloupka et al. 1993). Phenanthrene was among the prior PAHs detected in the sediments in campaign 2011. It was also quantified in sediments of the hot-spot sites during campaign May 2013, which were sampled in parallel to the fish that exhibited genotoxic effects and significant hepatic EROD induction. Phenanthrene occurred with 9% of PAH$_{216}$ (28 ± 8 ng/g) at Chongqing and with 13% of PAH$_{216}$(44 ± 30 ng/g) at Kaixian, while nitrate – a nitrite precursor – was detected in concentrations of 7.4 mg/L in the Yangtze water at Chongqing at the same time (Wolf 2015), thus making Nitro-PAHs potential candidates for the detected effects.

Further, also heterocyclic PAH derivatives (NSO-Het) are known EROD inducing AhR agonists (Hinger et al. 2011) and to be among the major CYP1A inducing compounds in sediment from a site strongly contaminated by chemical industry at Bitterfeld, Germany (Brack
& Schirmer 2003). The have also been found to induce genotoxic effects in fish (Shailaja et al. 2006a, Brinkmann et al. 2014). Creosote contaminated industrial areas are considerable sources for heterocyclic PAHs, which are more mobile – and thus more bioavailable - than homoeyclic PAHs with respect to their higher water solubility (Blum et al. 2011, Hinger et al. 2011, Brinkmann et al. 2014). Further investigations are required to verify these presumptions.

A promising method to supplement the current study is bioassay-guided effect directed analysis (Brack & Schirmer 2003, Brack et al. 2005, Hecker & Hollert 2009). This concept is based on the fractionation of complex samples according to physico-chemical properties, like polarity, and the investigation of the resulting sub-samples in bioassays, in order to identify the active fractions and the containing causative agents (cf. Fig. III-2.12). This step has been performed with selected samples of this study and will be described by Xiao (2016).

Fig. III-2.12. Conceptual approach of a study combining the triad approach (Chapman 1990) and effect-directed analysis (Brack 2003). Redrawn from Suares Rocha (2009).

2.6 Spatial patterns and relationship of endpoints

Spatial and temporal variations of several endpoints were recorded for sediment and the darkbarbel catfish P. vachellii, from various sampling sites in the TGR mainstream, major tributaries and its watershed and are combined in (Table III-2.9).

Comparing the individual endpoints, significant correlations could be found between chemical analysis and the in vitro EROD and in vivo FET, which were all assessed with the sediment
extracts (cf. Chapters III-2.4.4 and III-2.4.6). Although mutagenicity was detected at all sites a higher burden with PAHs did not necessarily result in stronger mutagenicity (Table III-2.9) (cf. Chapters III-2.2.1, III-2.3.2, III-2.3.5, III-2.3.6).

However, after the sediment assessment in September 2011 more pronounced biomarker responses in the bottom-dwelling fish have been expected to be found in May 2012, for example at Yunyang, which was comparable to Chongqing and Kaixian in several endpoints (Table III-2.9). It has to be considered that the situation in 2011 does not necessarily reflect the situation in 2012, although biliary PAH metabolites could be determined in fish from all sites. The only consistency was observed for the sites Chongqing and Kaixian, which were therefore further investigated in May 2013 for a more detailed and combined assessment of sediment and fish.

Both sites revealed overall lower PAH concentrations in the sediments compared to 2011, but more pronounced biomarker responses (Table III-2.9). Genotoxic impacts were particularly observable at Kaixian in 2012 and 2013, whereas at Chongqing only in 2013 (cf. Chapter III-2.4.1). Hepatic EROD induction was particularly detectable at Chongqing in both years (Table III-2.9). The identification of Chongqing city as site of particular significance in the TGR area makes sense, due to its enormous population and industrial importance. The conspicuity of the artificial Hanfeng Lake at Kaixian may be regarded to a lesser dilution of discharged contamination compared to the TGR mainstream, and a potential accumulation of it in the lower section of the lake.

Except those two “hot-spot sites” – that displayed a burden with contaminants in combination with inductions in vitro, in vivo and in situ - no clear pattern could be observed among the endpoints. Pollution patterns of PAHs at the different locations per site – that are also partly reflected by the correlated in vitro EROD induction and embryotoxic/teratogenic effects (cf. Chapter III-2.3.5) - and their emission sources (cf. Chapter III-2.2.5). Bioavailability of the pollutants, non-target compounds, as well as synergistic and antagonistic effects of the contaminants, in addition to uptake, metabolism and secretion pathways in the fish corroborate a direct correlation between the different endpoints.

Since P. vachellii has proven to be a suitable monitoring species and the selected endpoints to be applicable, a continuous monitoring applying a comprehensive strategy - combining sediment, water and biota - is strongly suggested to verify the reported observations and clarify the open questions.
Table III-2.9. Overview of the temporal and spatial variations of different endpoints in sediment and fish at the respective sampling campaigns September 2011 (I; sediment), May 2012 (II; fish), and May 2013 (III; sediment and fish) given as means in relation to the reference site (REF). Symbols represent relative content/induction compared to the reference: - = <2 fold, + = 2 to 4 fold, ++ = 4 to 6 fold, +++ = 6 to 8 fold, ++++ = 8 to 10 fold, +++++ = > 10 fold.

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<sup>a</sup>PAH<sub>210</sub> concentration (Chapter III-2.2.1); <sup>b</sup> In vitro EROD activity (bio-TEQ EC25 values) (Chapter III-2.3.3);<br> <sup>c</sup> Embryotoxic/teratogenic effects (EC50 values) (Chapter III-2.3.4); <sup>d</sup> Mutagenicinity (number of revertants at test concentration 100 mg/mL relative to respective negative control) (Chapter III-2.3.2); <sup>e</sup> Biliary 1-OHPyr content (Chapter III-2.4.2); <sup>f</sup> Hepatic EROD activity (Chapter III-2.4.4); <sup>g</sup> Hepatic GST activity (Chapter III-2.4.4);<br> <sup>h</sup> Micronucleus rate in erythrocytes (Chapter III-2.4.1); <sup>i</sup> Showed cytotoxicity in highest tested concentration.
Chapter III – Results & Discussion

2.7 Conclusion

Under consideration of the implications the TGR faces since its impoundment - a progressive urbanization, industrialization, rising ship traffic and submersion of abandoned contaminated sites - the environmental situation during the sampling campaigns between September 2011 and May 2013 appeared to be less pronounced than expected. Although, the significant biological impairments at Chongqing and Kaixian suggest a toxicity reduction evaluation for these areas.

However, most parts seem not yet to exhibit ecotoxicological effects or only to a minor degree. The TGR appears to be in a comparable or even less contaminated state than other national or international water bodies, based on detected pollution concentrations in sediment and water. As immense amounts of water and sediment are discharged into and carried by the Yangtze River, this observation may be primarily contributed to a strong dilution effect. This leads to lower concentrations and respectively less acute effects, but conceals the total chemical burden under consideration of a mass-balance approach. This means that a contamination problem of the Yangtze River is not solved, but simply relocated (compare Chapter III-1.5, Müller et al. 2008, Floehr et al. 2013). The mass balance approach for PAHs referred to the immense sediment influx suggested a deposition of 216-636 kg PAH/day (0.2-0.6 mg PAH/m²/day) (cf. Chapter III-2.2.1) and the combustion of fossil fuels appeared to play a significant role in the emission of PAHs into the environment along the reservoir. Thus, it can be concluded that air pollution, which plays a considerable role in China (Zhang & Tao 2009, Zhang et al. 2009b), also affects the quality of its water bodies. And as the PAHs pass several exposition pathways – air and water – they pose a risk to humans and wildlife already before they are deposited in the TGR. Compared to the external sources, like air pollution, it is suggested that the risk of internal pollution emission from the submerged sites plays only a minor role, due to the deposition of vast amounts of sediment in the TGR and the dilution effect.

Further, it could be shown in this study that the bioavailability of particle bound pollutants was rather low. However, hydraulic effects like frequently occurring flood events in the Yangtze River can increase the bioavailability through remobilization of pollutants to peak situations (Stachel et al. 2005, Feng et al. 2007b, Brinkmann et al. 2010, Wölz et al. 2010). In the same course particle bound pollutants can be relocated on agriculturally used areas of the TGR’s water fluctuation zone, which covers an elevation range of about 30 m from 145 m to 175 m altitude (a.s.l.). Therefore, the importance of sediments as sink and source for pollutants in the TGR needs to be considered.
Chapter III – Results & Discussion

Especially with respect to mutagenic properties of pollutants low concentrations cannot directly be set equal to low risk, as threshold values are under discussion due to the mutagenic mode of action. The mutagenicity of sediment extracts and genotoxic impacts on the fish at particular sites along the reservoir and in its watershed have been demonstrated. This study supports the importance of mutagenic and genotoxic impacts for the quality of Chinese water bodies, e.g., as source for drinking water (Shu et al. 2002, Lv et al. 2015), and recommend their inclusion into routine monitoring programs as suggested before by Wu (2005) for marine systems.

The individual outcomes of this study and the crosslinks between chemical analysis, in vitro, in vivo and in situ investigations support the important role of AhR-mediated adverse impacts. Further, it supports the role of PAHs as key pollutants in the TGR area, as also shown in other studies (Wang et al. 2009, Wang et al. 2013, Wolf et al. 2013a). As Hong et al. (2012) emphasized that urbanization effects on the environment can be recorded by PAHs in sediment, are PAHs hereby suggested as suitable marker compounds or indicators for monitoring of the reservoirs sediment pollution status. This is in accordance with Wang et al. (2013), who stated the same for the reservoirs water.

However, only a certain amount of the recorded activities could be explained by the presence of PAHs. Thus, the focus should be extended further to non-target pollutants - like unsubstituted, nitro- and heterocyclic PAH derivatives and other important dioxin-like compounds - to identify more causative agents. In order to do so, bioassay-guided effect directed analysis has been proven to be a valuable tool (Brack & Schirner 2003, Brack et al. 2005, Hecker & Hollert 2009, Xiao 2016). It has also to be considered that only organic pollutants were in the scope of this study. Inorganic contaminants, like heavy metals or high nitrogen levels due to excessive regional fertilization, as well as other biotic and abiotic factors, like pathogens or oxygen depletion due to eutrophication by repeatedly occurring algal blooms, may also play their part as detrimental factors. A complex variety of pathways exist that influence the reservoirs biota.

The obtained results demonstrate that pollution has an effect on the fish of the TGR area. As a burden with PAH metabolites, in addition to a number of effects, like genotoxicity and Ah receptor mediated changes in metabolism, could be recorded in the fish from the identified hot-spot sites Chongqing and Kaixian. In this course, the chosen monitoring fish species Pelteobagrus vachellii, hence was proven to be well suited for biomonitoring, particularly due to its wide distribution along the TGR and other sections of the Yangtze River. The biomarkers in combination with the chemical analysis from this study and water data from other studies
Chapter III – Results & Discussion


Therefore, habitat destruction due to hydrological alterations of the TGR area and overfishing are considered to be the primary triggers of a fish decline in this region, as reported by Chen et al. (2009). With pollution currently rather playing a rather sublethal role, as an additional risk factor that promotes disorders, like cardiovascular diseases and cancer, and with an influence on the overall fitness of the fish. The shift in species composition and age structure with a decrease of adults towards younger fish, as described by Chen et al. (2009), can more likely be regarded to the altered hydrologic conditions, habitat fragmentation and shrinkage, as well as to resources overexploitation, respectively (Liu et al. 2005, Chen et al. 2009).

Especially fish at early life stages are very sensitive to contamination, as demonstrated for AhR-mediated activities and PAHs (Walker & Peterson 1991, Guiney et al. 1997, Le Bihanic et al. 2014). An interference of sublethal chronic exposure during this vulnerable stage may even lead to impairments that evolve only in the long-term (Hicken et al. 2011). Chronic exposure to lower doses of pollutants can evoke detrimental impacts on physiological, immunological and behavioral processes, consequently reducing the overall fitness and susceptibility to pathogens (Mason 2001). The suggested rather poor condition of fish, may therefore also be regarded to an influence of sublethal pollution.

The complex situation in the field and the difficulty to predict environmental hazards solely from chemical data or bioassay studies call for a holistic environmental assessment. This should include *in situ* approaches with higher organismic levels due to a greater ecological relevance than isolated *in vitro/vivo* laboratory studies, which yet in their turn allow a faster screening of environmental samples and help to identify modes of action. Those should be combined with chemical screening to deliver potential suspects for the role of the inducers. The combined application of these methods with a variety of endpoints and biomarkers was proven to be applicable and successful in this study.

The recent study should represent a foundation for further monitoring programs of the area. And as an examination of the reservoirs status in the early days after its impoundment it should constitute a reference for the further development of the reservoirs environmental condition in course of proceeding urbanization and industrialization. It should help to initiate and enforce necessary countermeasures, like improved wastewater treatment and emission standards, in time to prevent environmental degradation in the long-term and sustain this unique ecosystem.
-Part 3-

PAH metabolism in immune organs

This chapter has been published as part of an article in a peer-reviewed journal:

Chapter III – Results & Discussion

3 PAH metabolism in immune organs

3.1 Abstract

Polycyclic aromatic hydrocarbons (PAHs) are immunotoxicants in fish. In mammals, phase I metabolites are believed to be critically involved in the immunotoxicity of PAHs. This mechanism has been suggested for fish as well. The present study investigates the capacity of immune organs (head kidney, spleen) of rainbow trout, Oncorhynchus mykiss, to metabolize the prototypic PAH, benzo[a]pyrene (BaP). To this end, we analyzed (i) induction of enzymatic capacity measured as ethoxyresorufin-O-deethylase (EROD) activity in immune organs compared to liver, (ii) the organ profiles of BaP metabolites generated in vivo, and (iii) rates of BaP metabolite production determined with microsomes in vitro. All measurements were done for control fish and for fish treated with an intraperitoneal injection of 15 mg BaP/kg body weight. In exposed trout, liver, head kidney and spleen contained similar levels of BaP, whereas EROD induction differed significantly between the organs, with liver showing the highest induction factor (132.8 times), followed by head kidney (38.4 times) and spleen (1.4 times). Likewise, rates of microsomal metabolite formation experienced the highest induction in the liver of BaP-exposed trout, followed by head kidney and spleen. Microsomes from control fish displayed tissue-specific differences in metabolite production. In contrast, in BaP-exposed trout microsomes of all organs produced the potentially immunotoxic BaP-7,8-dihydrodiol as the main metabolite. The findings from this study show that PAHs like BaP are distributed into immune organs of fish and they provide the first evidence that immune organs possess inducible PAH metabolism leading to in situ production of potentially immunotoxic PAH metabolites.
3.2 BaP exposure of rainbow trout: Verifying effectiveness of treatment

To obtain an indication whether the experimental BaP exposure was effective in inducing BaP metabolism in the liver as the main metabolic organ, we analyzed hepatic EROD activity in control and BaP-exposed fish. The fish injected with 15 mg/kg BaP and sampled 5 days after injection showed a 133 times induction of hepatic EROD levels over controls. In addition, we measured bile fluorescence by means of fixed wavelength fluorescence spectroscopy. This method, which is based on the fact that PAH metabolites are fluorescent, is frequently used as a summary parameter to indicate that the liver actively performs PAH metabolism and metabolite secretion. In control fish, 1 µL of bile displayed a mean fluorescence of 2,799 ± 812 fluorescence units (fu) (n=5), whereas the same volume of bile from BaP-exposed fish showed a mean fluorescence of 120,698 ± 29,833 fu (n=5), which corresponds to a 43 fold increase of fu.

3.3 EROD activity in head kidney and spleen of control and BaP-exposed rainbow trout

EROD activity, which is executed by the PAH-metabolizing CYP1A, was used as proxy to assess (i) whether head kidney and spleen principally possess enzymatic capacity for PAH metabolism, (ii) how it compares to the reference metabolic organ, the liver, and (iii) whether the enzymatic capacity of spleen and head kidney for PAH metabolism is inducible by BaP exposure. Microsomes prepared from the immune organs head kidney and spleen of control fish showed EROD activities of 0.25 ± 0.016 and 0.48 ± 0.085 pmol resorufin/mg protein/min, respectively (Fig. III-3.1). This corresponded to 3 % and 5.6 %, respectively, of the EROD activity of in the liver of control trout. Treatment of trout with 15 mg BaP/kg body weight resulted in increased microsomal EROD activities in both immune organs: 9.6 ± 0.22 pmol resorufin/mg protein/min in head kidney, and 0.65 ± 0.29 pmol resorufin/mg protein/min in spleen. These levels correspond to 0.8 % and 0.06 % respectively, of EROD activity in the liver of BaP-exposed fish. Induction factors were higher in the liver (132.8) than in head kidney (38.4) and spleen (1.4). The treatment-related increases of EROD activities were significant in the liver and head kidney, but not in the spleen. Differences between microsomal EROD levels in liver and immune tissues of BaP-exposed fish were more pronounced than in control fish, with hepatic EROD activities being 119 times higher than EROD activities in the head kidney, and 1758 times higher than in the spleen.
3.4 Levels of BaP and BaP metabolites in head kidney, spleen, liver, and bile of rainbow trout in vivo: Tissue analysis

Liver (n=5), head kidney and spleen tissues (n=3, respectively) from control and BaP-exposed trout were extracted and analyzed for the presence of BaP and BaP metabolites. Additionally, bile of control and exposed fish was analyzed. Data is presented as absolute numbers (ng substance/mg tissue or μL bile, Fig. III-3.2).

Control fish had neither detectable BaP concentrations nor detectable metabolite concentrations. In exposed fish, the parent compound BaP was detected in all three organs. In BaP-treated fish, the highest amount of BaP was found in the head kidney (1.3 ng/mg ± 1.1), followed by the liver (0.4 ng/mg ± 0.48) and the spleen (0.25 ng/mg ± 0.16). Regarding the total sum of BaP metabolites, there was a different ranking of the three organs (Fig. III-3.2). Most metabolites were present in the liver (0.38 ng/mg ± 0.2), followed by the spleen (0.017 ng/mg ± 0.0072). In the head kidney, none of the analyzed BaP metabolites was found. The sum of all analyzed metabolites plus BaP (in the following referred to as “total substance level”) was 0.78 ± 0.4 ng substance/mg tissue in liver, 0.27 ± 0.2 ng substance/mg tissue in spleen and 1.3 ± 1.1 ng substance/mg tissue in head kidney. In bile, 13.27 ± 2.8 ng substance/μL were present. Total substance levels of head kidney, spleen and liver tissue were not significantly different.
HPLC analysis of liver tissue showed that the major hepatic metabolites were 3-hydroxy-BaP (0.27 ± 0.19 ng/mg tissue) and BaP-7,8-dihydrodiol (0.1 ± 0.05 ng/mg), a precursor to BPDE (Fig. III-3.2). Furthermore, BaP-9,10-dihydrodiol (0.0013 ± 0.0014 ng/mg) and 9-hydroxy-BaP (0.005 ± 0.0035 ng/mg) were present in lower amounts. BaP-4,5-dihydrodiol was not detected, neither in the liver nor in any other tissue or in bile. The bile displayed a similar metabolite distribution pattern as the liver, with BaP-7,8-dihydrodiol and 3-hydroxy-BaP being the main metabolites (6.1 ng/μL ± 1.7 and 6 ng/μL ± 1.8, respectively), while 9-hydroxy-BaP was present only at low concentrations (0.3 ng/μL ± 0.07). Neither BaP-9,10-dihydrodiol nor BaP could be detected in the bile.

The spleen had a metabolite profile different to the liver (Fig. III-3.2). Whereas we detected BaP-dihydrodiols, neither 3-hydroxy-BaP nor 9-hydroxy-BaP was present. The main BaP metabolite in the spleen was BaP-7,8-dihydrodiol (0.02 ng/mg ± 0.007), whereas BaP-9,10-
dihydrodiol was present at a concentration that was one order of magnitude lower (0.002 ng/mg \pm 0.0008). In the head kidney, no metabolites were found (Fig. III-3.2).

For each of the three tissues, the ratios of metabolites and BaP concentrations were estimated. To this end, metabolite and BaP concentrations were converted from ng substance into pmol to account for the differences in molecular weight. The sum of BaP and metabolite concentrations (in pmol/mg tissue) in each tissue was taken as 100% and the metabolite concentrations expressed as percent of this sum (Table III-3.1). This calculation shows that the liver tissue contained a clearly higher percentage of metabolites than the two immune organs: while in the liver, the sum of BaP plus metabolites contained 46.6 % metabolites, the metabolites contributed only 7.2 % and 0 % in spleen and head kidney, respectively.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Liver [%]</th>
<th>Liver [pmol]</th>
<th>Spleen [%]</th>
<th>Spleen [pmol]</th>
<th>Head Kidney [%]</th>
<th>Head Kidney [pmol]</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-OH</td>
<td>34</td>
<td>1.01</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>7,8-diol</td>
<td>11.8</td>
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<td>n.d.</td>
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<tr>
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<td>0.0046</td>
<td>0.7</td>
<td>0.007</td>
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<td>n.d.</td>
</tr>
<tr>
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<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Total metabolites</td>
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<td>7.2</td>
<td>0.077</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>BaP plus metabolites</td>
<td>100</td>
<td>2.97</td>
<td>100</td>
<td>1.07</td>
<td>100</td>
<td>5.1</td>
</tr>
</tbody>
</table>

n.d. = not detected

3.5 Rates of BaP metabolite formation in the head kidney, spleen and liver of rainbow trout: *In vitro* microsomal incubations

Microsomes prepared from liver, head kidney and spleen of untreated and BaP-exposed trout were incubated with BaP to analyze *in vitro* rates of BaP metabolite formation and metabolite patterns. Microsomal preparations of all three organs were capable to metabolize BaP, both in control fish and in BaP-exposed fish (Fig. III-3.3). Liver microsomes of untreated fish produced 3-hydroxy-BaP (106.8 ng/mg protein/h), BaP-7,8-dihydrodiol (68.1 ng/mg protein/h), 9-hydroxy-BaP (27.9 ng/mg protein/h) and BaP-9,10-dihydrodiol (4.1 ng/mg protein/h).
protein/h). Liver microsomes of BaP-treated fish produced the same metabolites, but at higher levels and in a different order: 1245.1 ng/mg protein/h of BaP-7,8-dihydrodiol, 602 ng/mg protein/h of 9-hydroxy-BaP, 572.7 ng/mg protein/h of 3-hydroxy-BaP and 238.6 ng/mg protein/h of BaP-9,10-dihydrodiol (Fig. III-3.3).

Spleen microsomes from control fish produced BaP-7,8-dihydrodiol (12.2 ± 0.8 ng/mg protein/h) and 9-hydroxy-BaP (4.2 ± 6.0 ng/mg protein/h) but neither 3-hydroxy-BaP nor BaP-9,10-dihydrodiol. Spleen microsomes from exposed fish produced BaP-7,8-dihydrodiol (20.7 ± 10 ng/mg protein/h), 9-hydroxy-BaP (8.6 ± 0.2 ng/mg protein/h) and BaP-9,10-dihydrodiol (2.4 ± 3 ng/mg protein/h). The difference of BaP-7,8-dihydrodiol and 9-hydroxy-BaP levels in microsomal incubations from control and BaP-treated fish was not significant. 3-hydroxy-BaP was not formed in any spleen incubation in detectable amounts.

Head kidney microsomes of control fish produced 3-hydroxy-BaP (13.9 ± 19 ng/mg protein/h) and BaP-7,8-dihydrodiol (9.9 ± 0.2 ng/mg protein/h), but neither 9-hydroxy-BaP nor BaP-9,10-dihydrodiol. In head kidney microsomes from BaP-exposed fish, the metabolite formed at the highest rate was BaP-7,8-dihydrodiol (72.7 ± 2.3 ng/mg protein/h), followed by 3-hydroxy-BaP (53.9 ± 17.8 ng/mg protein/h). In contrast to control fish, head kidney microsomes of BaP-treated fish produced also 9-hydroxy-BaP (33.1 ± 8.3 ng/mg protein/h) and BaP-9,10-dihydrodiol (13.1 ± 1.5 ng/mg protein/h). The formation of BaP-7,8-dihydrodiol was increased significantly in microsomes of BaP-exposed fish over control microsomes.

When summing up the metabolic rates of the individual metabolites for each organ, rates of liver microsomes were found to be one to two orders of magnitude higher than in head kidney and spleen. The sum of metabolites produced by liver microsomes of control fish was 206.9 ng substance/mg protein/h, whereas head kidney showed a total rate of 23.9 ± 19.5 ng substance/mg protein/h and spleen 16.5 ± 6.8 ng substance/mg protein/h. In microsomes of BaP-treated fish, total metabolite formation rates showed the same ranking: they were highest in the liver (2658.4 ng substance/mg protein/h), followed by head kidney (172.8 ± 22.2 ng substance/mg protein/h) and spleen (31.7 ± 13.9 ng substance/mg protein/h). None of the microsomal preparations formed BaP-4,5-dihydrodiol. Induction of metabolic rates of control fish to BaP-treated fish was highest in the liver (12.9 times) followed by head kidney (7.2 times) and spleen (1.9 times). The treatment-related increases of total metabolite formation were significant in liver and head kidney, but not in the spleen. One unknown peak occurred both in spectra from BaP treated liver and head kidney incubations at 35-36 min, which was not present in any control incubation.
Fig. III-3.3. BaP formation of (A) liver (n = 1), (B) spleen (n = 2) and (C) head kidney (n = 2) microsomes. Microsomes of both BaP-treated (5 days after injection of 15 mg BaP/kg body weight) and control fish were incubated with BaP for 1 hour. Afterwards, the reaction was stopped and BaP metabolites were extracted and analyzed using HPLC. Values are given as mean ± SD. * = p < 0.05; ** = p < 0.001, significantly different from control group. The data of liver microsomal incubations was not applied for statistical analysis.
Table III-3.2. Relative distribution of benzo[a]pyrene (BaP) metabolites in the liver, spleen, and head kidney microsomes of control and untreated trout. The sum of metabolites that were extracted from microsomal incubations correlates to 100%. Picomoles give amount of picomoles of substance per milligram of protein per hour.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Liver</th>
<th></th>
<th>Spleen</th>
<th></th>
<th>Head Kidney</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>BaP-treated</td>
<td>Control</td>
<td>BaP-treated</td>
<td>Control</td>
<td>BaP-treated</td>
</tr>
<tr>
<td>3-OH</td>
<td>[%]</td>
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<td>n.d.</td>
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</tr>
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<td>n.d.</td>
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</tr>
<tr>
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<td>[%]</td>
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<td>[pmol]</td>
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<td>4.4</td>
<td>0.04</td>
<td>0.073</td>
<td>0.035</td>
</tr>
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<td>9,10-diol</td>
<td>[%]</td>
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<td>8.7</td>
<td>n.d.</td>
<td>7.5</td>
<td>n.d.</td>
</tr>
<tr>
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<td>[pmol]</td>
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<td>0.009</td>
<td>n.d.</td>
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<td>9-OH</td>
<td>[%]</td>
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<td>23.4</td>
<td>26.9</td>
<td>28.4</td>
<td>n.d.</td>
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<tr>
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<td>0.016</td>
<td>0.03</td>
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<tr>
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<td>[%]</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>[pmol]</td>
<td>0.75</td>
<td>9.5</td>
<td>0.056</td>
<td>0.112</td>
<td>0.085</td>
</tr>
</tbody>
</table>

n.d. = not detected

3.6 BaP metabolites in the head kidney, spleen, and liver of rainbow trout: Comparison of metabolite patterns from tissue analysis and microsomal incubations

In the liver of control fish, ranking of metabolites on the basis of their tissue concentrations was 3-hydroxy-BaP > BaP-7,8-dihydrodiol > 9-hydroxy-BaP > BaP-9,10-dihydrodiol. The rate of metabolite formation by microsomes of control liver showed an identical ranking, i.e. the rates were highest for 3-hydroxy-BaP, followed by BaP-7,8-dihydrodiol, 9-hydroxy-BaP and BaP-9,10-dihydrodiol. In contrast, liver tissue and microsomes from BaP-exposed fish differed in their ranking, with microsomes producing BaP-7,8-dihydrodiol as main metabolite while 3-hydroxy-BaP was the dominant metabolite in the intact liver. The main metabolite in the spleen of control and BaP-exposed fish, was BaP-7,8-dihydrodiol and spleen microsomes also showed the highest rate of formation for this metabolite. The second highest metabolic rate in spleen microsomes from control and BaP-exposed fish were observed for 9-hydroxy-BaP, but this metabolite was not found in spleen tissue in vivo. Differences between the results of the microsomal incubations and tissue analysis were prominent in the head kidney, where no metabolites at all were found in the intact tissue, whereas microsomes generated BaP metabolites. Head kidney microsomes from BaP-exposed fish produced all four metabolites,
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with the ranking of BaP-7,8-dihydrodiol > 3-hydroxy-BaP > 9-hydroxy-BaP > BaP-9,10-dihydrodiol. Head kidney microsomes of control fish produced 3-hydroxy-BaP at the highest rate, followed by BaP-7,8-dihydrodiol. The relative importance of the individual metabolites in microsomes from liver, head kidney and spleen, expressed as percent of total metabolite levels, is shown in Table III-3.2.

3.7 Discussion

In mammals, the importance of tissue-specific PAH metabolism for organ-related adverse effects is well known (Miller & Ramos 2001, Ioannides & Lewis 2004). The capability of mammalian immune organs to generate PAH metabolites is thought to be critically involved in PAH immunotoxicity (Burchiel & Luster 2001, Ioannides & Lewis 2004), and tissue or cell-dependent differences in amount or spectrum of the produced metabolites can greatly influence the toxicological outcome. Also for fish, it has been shown that tissue-specific PAH metabolism is an important determinant in PAH toxicity - e.g., James et al. (1997) -, as it has been suggested with respect to PAH immunotoxicity (Carlson et al. 2004a, Reynaud & Deschaux 2006, Reynaud et al. 2008). However, the actual capability of piscine immune organs to metabolize PAHs has not been demonstrated to date. Here, we investigated whether immune organs of rainbow trout have the capacity for in situ metabolism of PAHs and whether metabolism is inducible by exposure to BaP.

The findings from the in vivo part of this study indicate that distribution of the parent compound, BaP, to the immune organs appears to be similar to distribution to the liver, as BaP concentrations in head kidney and spleen tissue were not significantly different from liver. Given the location of fish immune organs in the vascular system, and their blood-filtering role (Press & Evensen 1999), substantial exposure of immune organs to circulating xenobiotics is to be expected. In fact, Valdez Domingos et al. (2011) showed in a toxicokinetic study on PAH distribution in medaka that a substantial fraction is distributed to the immune organs. Medaka head kidney tended to accumulate higher amounts of PAH than the spleen (Valdez Domingos et al. 2011), what is in agreement with our observation on rainbow trout. The difference between the two immune organs might be related to their role and position in the blood system of fish: while the head kidney receives a direct supply from the dorsal aorta close to its origin, the more distant location of the spleen as well as its function as an encapsulated plasma filter imply lower blood flow rate and supply. As shown by Barron et al. (1987) for rainbow trout, the kidney receives a clearly higher blood flow distribution (measured as percent of cardiac output) and organ perfusion (mL blood per gram of tissue per hour) than does the spleen.
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Do the immune organs of rainbow trout possess a capacity for BaP metabolism, and is it inducible? We approached this question by analyzing rates of BaP metabolite formation by microsomes prepared from head kidney and spleen of control and BaP-exposed trout. These rates of the immune organs were compared to rates of BaP metabolite formation in liver microsomes as a benchmark. In parallel, we measured microsomal EROD activity - as proxy of the overall enzymatic capacity of a tissue for BaP metabolism - in the immune organs and the liver.

In control fish, liver microsomes showed significantly higher rates of BaP metabolite formation, and higher EROD activity than the immune organs. The head kidney possessed 3 % of the hepatic EROD activity and the metabolite formation rate of the microsomes was 12 % of the hepatic one. With these activities, the head kidney is clearly less active than trout liver, but also less active than the excretory kidney of trout (Pesonen et al. 1987). The spleen reached 8 % of the metabolite formation rate of the liver and had 5.6 % of the hepatic EROD activity. After BaP exposure, levels of BaP metabolites were highest in the liver, and liver microsomes displayed the strongest induction in the rate of metabolite production and of EROD activity. In the head kidney of BaP-exposed fish, microsomal metabolite levels reached 7 % of liver metabolite levels, BaP metabolism rates were accelerated by a factor of 7.2, and EROD activity increased by a factor of 38.4. The spleen of BaP-exposed fish contained BaP metabolites at 1 % of the hepatic level, its EROD activity was elevated by a factor of 1.4, and the rates of microsomal metabolite formation were increased by a factor of 1.9. Notably, whereas BaP treatment led to a significant increase of metabolite production rates in liver and head kidney, it failed to do so in spleen. This was paralleled by EROD activities, which increased significantly in liver and head kidney of BaP-exposed fish, but not in spleen. The main conclusions from these findings are that (i) the immune organs possess metabolic capacity for BaP transformation, (ii) there exists good agreement between the tissue levels of EROD activity and the rates of BaP metabolite formation, (iii), the metabolic capacity is inducible in the head kidney but not in the spleen, and (iv) that the metabolic capacities of the immune organs, both in control and BaP-exposed trout, are clearly lower than in the liver.

Does the BaP metabolite spectrum obtained in the present study from liver and immune organ microsomes agree with published data on BaP metabolites in fish? A comparison is possible only for the liver, as almost no information exists on metabolite spectra of other organs. For this organ, our findings largely agree with literature reports. BaP-incubation of hepatic microsomes or isolated hepatocytes were reported to produce BaP-9,10-dihydrodiol, BaP-7,8-
dihydrodiol, 9-hydroxy-BaP and 3-hydroxy-BaP (Stegeman et al. 1984, Sikka et al. 1990, Yuan et al. 1997). A more controversial case appears to be BaP-4,5-diol. In our study, this metabolite was not detected, neither in the microsomal incubations nor in the tissues. In contrast, Williams and Buhler (1984), using purified cytochrome P450 LM4a and LM4b (CYP1As) from rainbow trout liver, observed the conversion of BaP to several quinones, BaP-3-OH, BaP-9-OH, BaP-7,8-diol, BaP-9,10-diol as well as BaP-4,5-diol. Interestingly, Miranda et al. (2006) when studying BaP metabolism in trout liver microsomes also did not observe the production of BaP-4,5-diol. Non-production of BaP-4,5-diol by fish tissues was reported by other authors as well (Stegeman et al. 1984, Yuan et al. 1997). Miranda et al. (2006) suggested that BaP-4,5-diol might be detectable in microsomal preparations only if huge amounts of microsomes are used and the products are concentrated prior to HPLC analysis.

In addition to analyzing BaP metabolites in microsomal preparations, which allows a determination of the rate of metabolite formation, we also analyzed BaP metabolites in the intact tissues of exposed fish in order to examine whether we would find the same type of metabolites as in the microsomal assays. Good agreement between tissue analyses and microsomal assays existed in the case of liver and spleen. In both organs, the same metabolite spectrum was observed in the microsomal assays and in the tissues, with the levels of metabolite being substantially lower in the spleen than in the liver. However, a discrepancy was observed for the head kidney: although the microsomal assays clearly indicated the capacity of this tissue to produce BaP metabolites, the head kidney of BaP-exposed fish contained no detectable levels of BaP metabolites. This is particularly surprising in comparison to the spleen, which contained BaP metabolites, despite significantly lower rates of BaP metabolite formation and EROD activity. Therefore, the absence of BaP metabolites from the head kidney in vivo cannot be explained by a lack of metabolic capacity of this tissue. We speculate that, due to the higher blood perfusion of the head kidney compared to the spleen (Barron et al. 1987, Schultz et al. 1999), metabolites are rapidly removed via the blood stream, so that the metabolite concentrations remaining in the tissue were below the detection limits of our analytical method.

With respect to the immunotoxicity of PAHs, it is not only important to know if the immune organs are capable of in situ PAH metabolism, but also if they produce a metabolite spectrum specific to these organs. Organ differences in BaP metabolite patterns might be related to tissue-specific expression of CYPs. Here, we studied only CYP1A, but multiple CYP forms participate in BaP metabolism, including CYP1A1, CYP1A2, CYP1B1, and several members of the CYP2 subfamilies and CYP3A4 (Bauer et al. 1995, Gautier et al. 1996, Scornaienchi et al. 2010). The
activities of these enzymes can vary between organs and even between specific immune cell types (Okano et al. 1979, Baron et al. 1998, Kapitulnik & Strobel 1999). For fish, the available database on tissue-specific expression of distinct CYP enzymes is limited. Best documented is the presence of CYP1A in the head kidney - e.g., Lorenzana et al. (1988), Pesonen et al. (1990) -, spleen (Taysse et al. 1998) or in distinct immune cell populations (Nakayama et al. 2008). Particularly the work in Buhler's group showed expression of a series of other CYP transcripts in the kidney of trout, mainly within the CYP1 and CYP3 families, both of which could be responsible for tissue differences in BaP metabolites - reviewed in Buhler and Wang-Buhler (1998). Tissue-specific differences of BaP metabolites, in fact, have been observed in the present study. For instance, 3-hydroxy-BaP was produced only by head kidney and liver but not by spleen microsomes. Importantly, during BaP exposure, liver and head kidney showed a shift in metabolite ranking from 3-hydroxy-BaP as main metabolite produced by control microsomes, to BaP-7,8-dihydrodiol as the main metabolite formed by microsomes of BaP-treated fish. As discussed above, this may indicate a BaP-induced alteration of the relative expression of CYP isoenzymes. Similar results were obtained in turbot (Telli-Karakoc et al. 2002). The dominant formation of BaP-7,8-dihydrodiol under BaP-exposure is toxicologically of interest. This metabolite suppresses T cell proliferation in mammals (Davila et al. 1996) and induces apoptosis in human B cells (Salas & Burchiel 1998). Further metabolism of this compound may lead to BPDE, an intermediate of BaP that binds to DNA and had immunosuppressive effects in murine splenocytes (Kawabata & White Jr. 1989). In medaka, a single intraperitoneal injection of BaP led to immunosuppressive effects in kidney lymphocytes that were not observed after concurrent exposure to BaP and ANF – a finding that points to a role of metabolism in the immunotoxic effects of BaP (Carlson et al. 2004a). This interpretation is corroborated by the finding of the present study that BaP-7,8-dihydrodiol, which is a known immunotoxic compound, is the dominant metabolite in the immune organs of BaP-exposed trout.

3.8 Conclusion

In conclusion, this study provides an answer to an important gap in our understanding of PAH immunotoxicity in fish. It has been suggested that the production of PAH metabolites in the immune cells is a mechanism through which PAHs cause immunotoxicity, but it had not been demonstrated yet whether immune organs indeed are capable of PAH metabolism. This study now provides strong evidence that rainbow trout immune organs possess an (inducible) capacity for in situ metabolism of the prototypic PAH, BaP.
Chapter IV

Overall Summary & Discussion
Chapter IV – Overall Summary & Discussion

1 Overall Summary & Discussion

In the beginning, the question “Dammed and damned?” was posed with regard to the unknown status of ecotoxicological impacts of organic pollution on the dammed Yangtze Three Gorges Reservoir section. As reviewed in Chapter III-1 only little research was done on the TGR after impoundment, which remained more or less a “black box” so far. Chapter III-2 helped to elucidate this “black box” by a holistic ecotoxicological assessment of the area. The findings were supplemented by Chapter III-3, which gave an important insight on the role of PAHs – as identified key pollutant class in the Yangtze in general and the TGR in particular – with regard to immunotoxicity in fish. This chapter picks up and summarizes the most important findings from all chapters in order to embed and discuss the investigation of the TGR (Chapter III-2) in the general context of the Yangtze River (Chapter III-1), with a particular focus on the role of PAHs and possible implications resulting from them (inter alia Chapter III-3).

1.1 Spatial variation of performed research

As identified in Chapter III-1, the largest part of the reviewed studies on the Yangtze River focused on pollutant levels in water and sediment and comparably less research was performed on in vitro, in vivo and in situ effects. The investigations mainly concentrated on the Yangtze River between Chongqing and the East China Sea, while reaches upstream Chongqing were only little investigated. A particular focus was on the areas of Chongqing, Wuhan, Nanjing, Shanghai and the Yangtze Estuary. The areas of main interest were in comparison also the main contaminated sites. Despite its increasing importance only a small amount of published studies were available on the Three Gorges Reservoir after impoundment (cf. Chapter III-1.5).

This makes sense in so far as an increasing demographic, agricultural and industrial increasing gradient exists from the mountainous regions in the west to the coast in the east. In order to narrow the economic gap between the relatively backward but resource-rich western and the booming eastern areas the Chinese government initiated several strategies in order to stimulate the economy in the relatively poorer regions, inter alia the “Rise of Central China” and the “Great Western Development Strategy” (Lai 2002, Government press release - China 2006, Lu & Deng 2011, Li et al. 2012b). The Three Gorges Dam is part of these strategies in its role to satisfy the need for energy, to control floods, and to safeguard the regional and transregional water supply, e.g., by transportation of the water from the TGR to the water scarce north. Further, the elevated water level of the impounded reservoir realized an improved navigation
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of large container ships between Sandouping and Chongqing, which was not possible before, becoming an essential factor for the economic development of the TGR region (cf. Chapter I-3) (Lai 2002, Ponseti & López-Pujol 2006, Bergmann et al. 2011, Xinhua 2014). Thus, it is to be expected that with a proceeding economic development of Western China the importance of the TGR will grow. Therefore, it is crucial to register the state of contamination and possible effects on the river’s biota, as well as to understand the local conditions in order to prevent environmental deterioration, as observed in Wuhan section and the Yangtze Estuary (cf. Chapter III-1.5).

1.2 Organic pollution of water and sediment

1.2.1 Yangtze River

Along the reservoir the most investigated compounds were PAHs, PCBs and OCPs, while data about the presence of emerging pollutants in the Yangtze River are still scarce. Particularly PAHs were the dominant pollutant class upon all contaminants detected in the river. Available data on emerging pollutants showed considerable levels of PFCs and PBDEs in sediment, as well as PAEs and NP in water in some sections of the Yangtze River. Compared to the 1990s, the PAH pollution in the Yangtze River has dramatically increased due to anthropogenic activities and PCB contamination decreased due to a governmental prohibition of electronic waste import at the beginning of 2000s. In comparison to the other parts of the river Wuhan section and the Yangtze Estuary stood out by exhibiting stronger pollution than the other investigated sites (cf. Chapter III-1.2.5 and Chapter III-1.2.6).

1.2.2 Three Gorges Reservoir

The situation at the TGR section fits into the general picture. In water, PAHs were of primary interest besides PCBs and OCPs, with some studies on emerging pollutants (cf. Chapter III-1.2). Referred to the rest of the reservoir comparably higher concentrations of PAHs, PCBs and OCPs were detected in the reservoirs water near Chongqing city and the dam. However, they were still in a comparable or even lower range compared to other sites in the Yangtze River (cf. Chapter III-1.2 and Chapter III-2.2.1) (Wang et al. 2009, Liu et al. 2011a, Wang et al. 2013, Wolf et al. 2013a).

With regard to sediment of the TGR, only PAHs could be identified upon 54 organic compounds, including PAHs, PCBs, OCPs and PBDEs, in this study, which is the first study on sediment after impoundment, emphasizing their role as key pollutants of the area (cf.
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Chapter III-2.2.1). Compared to the PAH$_{16}$ levels at Yangtze Chongqing section (257-723 ng/g, mean: 359 ng/g) and the Jialing River (132-349 ng/g, mean: 240 ng/g) during the impoundment in October 2009 (Tang et al. 2011), detected levels of PAH$_{16}$ within the scope of this study were higher at Yangtze Chongqing section (1,653-816 ng/g, mean: 1,234 ng/g) and Jialing River (450 ng/g) in September 2011 after impoundment. During the campaign May 2013 they were in a comparable or even lower range in the Yangtze Chongqing section (150-433 ng/g, mean: 303 ng/g) and in the same range in the Jialing River (278-299 ng/g; mean: 289 ng/g). In comparison to other sections along the Yangtze River the sampled sediments in the TGR exhibited a similar PAH$_{16}$ content, but were mostly rather in the lower part of the ranges detected elsewhere (cf. Chapter III-1.2.1 and Chapter-III-2.2.1).

Judging the TGR’s water quality in international comparison, Wolf et al. (2013a) stated that out of 207 analyzed organic pollutants measured in the TGR after impoundment, including PAHs, PCBs, OCPs, PBDEs and PFCs, with exception of the two pesticides Picloram and Clopyralid, all other compound concentrations were in same range or even lower compared to surface waters in western industrialized countries. This stands in agreement with the investigation of the 54 compounds in surface sediment in this study, which were also comparable or lower than those detected in major Chinese and European rivers (cf. Chapter III-2.2.1).

1.3 Sources of contamination

1.3.1 General

In general, along the Yangtze River the predominant emission of PAHs originated from combustion sources, e.g., from coal, wood and petroleum as well as vehicle emission (cf. Chapter III-1.2.1).

1.3.2 Three Gorges Reservoir

The main contributors to PAH contamination in Chongqing section in the Upper Reaches of the Yangtze River were – based on the reviewed articles in Chapter III-1.2.1 – the combustion of coal in addition to wastewater discharges. The PAH source analysis of sediments from the TGR in this study also highlighted the primary impact of combustion sources (pyrogenic) on the more urbanized and industrialized upper TGR section – including Chongqing. The industrial emission, e.g., from coal combustion during power generation, as well as urban air pollution may have a serious influence on the reservoirs contamination status. Compared to this, the reservoirs mid-low section was rather dominated by petrogenic sources. A refined identification
of the combustion sources emphasized the importance of liquid fossil fuel combustion all along
the reservoir. Urban traffic emissions and runoff, as well as intensified shipping activities since
the impoundment of the reservoir, can be accounted to be the main contributors for this. These
observations are in accordance with the water analysis by Wang et al. (2009, 2013). The
investigated tributaries in the TGR were primarily affected by petrogenic sources, e.g., by oil
and fuel spillage from ships (cf. Chapter III-2.2.5).

1.3.3  Middle and Lower Reaches of the Yangtze River

The PAHs in sediments at Wuhan section in the Middle Reaches of the Yangtze River were
mainly caused by coal burning and petroleum combustion. Whereas PAHs found in the Yangtze
Estuary in the near-shore area in the Lower Reaches mainly derived from petroleum and
biomass (mainly coal) combustion, as well as vehicle emission; those detected in the farther
shore zone originated mainly from petroleum combustion of shipping processes and shore side
discharge (cf. Chapter III-1.2.1).

1.3.4  Atmospheric deposition

Concerning overall China, particularly biomass burning, domestic coal combustion and coke
ovens have been identified as significant sources for the atmospheric emission of PAHs (Zhang
et al. 2007, Zhang & Tao 2009).

With regard to other main organic pollutants along the Yangtze River atmospheric deposition
also played a crucial role. In addition to runoff from the land and wastewater those were the
most relevant input sources of PCBs. They primarily originated from former national
production and import of contaminated electronic devices. DDTs, HCHs, as well as
PCDDs/DFs as impurities, have originated from the usage of pesticides in the past decades. The
occurrence of OCPs in regions without prior application of DDT and HCH has been explained
by atmospheric transport and sedimentation (“cold condensation”). Moreover, atmospheric
deposition was a significant input source of PBDEs in the Yangtze Delta (cf. Chapter III-1.2).

This underlines the importance, interconnection and impact of air pollution on the quality of
China’s water bodies.
1.4 Water and sediment quality according to guideline values

1.4.1 Water

In general, the water quality of the Yangtze River met the guideline values of the Chinese national standard (Ministry of Environmental Protection - China 2002) and the European Water Framework Directive (EWFD Directive 2008/105/EC). Guideline values were exceeded only for PAHs (BaP as indicator) at Wuhan section and Nanjing/Jiangsu section (Ministry of Environmental Protection - China 2002, EWFD Directive 2008/105/EC), as well as for PCBs at Nanjing/Jiangsu section (Ministry of Environmental Protection - China 2002). Among the emerging pollutants, NP exceeded the water quality criteria at Chongqing section prior to the impoundment (EWFD Directive 2008/105/EC), and some PAEs at Chongqing section during impoundment, as well as Wuhan section and the Yangtze Delta (Ministry of Environmental Protection - China 2002, EWFD Directive 2008/105/EC) (cf. Chapter III-1.2.6).

With respect to the water quality of the TGR after impoundment, Wolf et al. (2013a) reported that out of the 207 analyzed organic pollutants, including PAHs, PCBs, OCPs, PBDEs and PFCs, all compounds with exception of the two pesticides Picloram and Clopyralid, met the standards for the Chinese National Drinking Water Quality Standard GB 5749 (Ministry of Health - China 2006) and the European Union Council Directive 98/83/EC on the quality of water intended for human consumption (The Council of the European Union 1998) and the EU Directive 2008/105/EC on environmental quality standards in the field of water policy.

1.4.2 Sediment

Regarding the sediment quality along the Yangtze River, concentrations of PAHs (BaP as indicator), PCBs and the pesticide HCB were below the “relevant contamination” according to the classification by the ICPR (2009), which is based on the EWFD (EWFD Directive 2008/105/EC) (cf. Chapter III-1.2.6). The same is true for the sediments from the TGR investigated in this study.

1.4.3 Problems with sediment quality guidelines

However, sediment quality guidelines are connected with the general problem to assess when sediments actually pose a risk to the environment. Sediment can act as sink and source of pollutants and particularly adsorb lipophilic compounds, which are thereby removed from the water column and thus less bioavailable. However, they can be remobilized again by
bioturbation, shipping activities, storms, currents and floods (cf. Chapter I-6). In addition, sediment quality cannot solely be judged based on chemical concentrations of pollutants. Additive, synergistic, antagonistic and masking interactions of analyzed substances and non-target compound mixtures further impede interpretations.

The ERL/ERM concept by Long et al. (1995) attempts to assess adverse biological effects of contaminants in sediments, inter alia with regard to compound mixtures. According to this concept ecotoxicological impacts were suggested in certain areas of the Yangtze River. The ERL values in sediments for total PAHs and PCBs were exceeded at Wuhan section and Yangtze Estuary, and DDT concentrations at all the sampling sites, indicating that “biological effects occasionally occur”. In these areas several impacts could be detected in vitro, in vivo and in situ (cf. Chapter III-1.2 and Chapter III-1.5).

1.5 In vitro/in vivo effect assessment of water and sediment

1.5.1 General

The in vitro/in vivo studies performed along the entire Yangtze River focused among measurable ecotoxicological endpoints especially on mutagenicity, genotoxicity and endocrine activity in water. They were also widely detectable along the river indicating a potential health risk in several areas. Although AhR-inducers, like PAHs and PCBs, appear to play a major role in the Yangtze River region only limited research was performed on biomarkers or bioassays displaying the cytochrome P450 activity (cf. Chapter III-1.3.4).

1.5.2 Three Gorges Reservoir

According to Qiu et al. (2003) and Shu et al. (2002), it has been found that prior to the impoundment of the TGR the water of the Yangtze River in the Upper Reaches at Chongqing and its tributary – the Jialing River – possessed mutagenic and endocrine potentials. Whereas the Jialing River appeared to be more seriously polluted than the Yangtze River. This was mainly attributed to agricultural, industrial and domestic discharges along the Jialing River, as well as a poor self-purification capacity compared to the Yangtze River (cf. Chapter III-1.3.4).

The investigation of sediment extracts from the TGR after impoundment in this study verified the mutagenic potentials in these two rivers, and also the higher inductions in the Jialing River. Mutagenicity could also be widely detected at the other sites in the TGR, particularly at Wushan. Potential sources for the mutagenic compounds in the strongest inducing samples have been referred to local industries (cf. Chapter III-2.3.2). Due to technical complications no
reliable results were achieved in measuring the endocrine activity in the sediment from the TGR in this study. Instead, AhR-mediated EROD induction could be measured in vitro at all sites, with significant inductions at Chongqing, Yunyang, Wushan and Kaixian compared to the reference site. In general, the activities appeared to be in the medium to upper range in national and international comparison (cf. Chapter III-2.3.3). Further, in vivo investigations showed that most sediment extracts did not induce significant mortality in the fish eggs, but numerous embryotoxic/teratogenic effects could be recorded that might result into severe consequences on the long run – particularly on the cardiovascular system (cf. Chapter III-2.3.4).

1.5.3 Middle and Lower Reaches of the Yangtze River

Water from the Yangtze River, Dong Freshwater Lake and the Yangtze feeding Han River at Wuhan section in the Middle Reaches showed mutagenic and endocrine activity as well. The tributary in this section possessed also a higher mutagenic potential than the mainstream (Dong et al. 2010). Alike the other sections, the Lower Reaches revealed mutagenicity and endocrine activity in the Yangtze River’s water at Nanjing and the Yangtze Estuary. According to mutagenicity the South Branch of the estuary seems to be more seriously polluted than the North Branch (Wu 2005) (cf. Chapter III-1.3.1).

1.6 Responsible compounds for in vitro/in vivo effects

1.6.1 Polycyclic aromatic hydrocarbons

The reviewed articles further showed that direct- and indirect-acting frameshift inducers were found to be the causative agents for mutagenicity in surface water of the Yangtze River at Chongqing (Shu et al. 2002), Wuhan section (Dong et al. 2010), Shanghai section (Shen et al. 2003a) and the Yangtze Estuary (Wu 2005) (cf. Chapter III-1.3.1). The same was found for the assessment of the sediment samples from the TGR in this study, where the causative agents have been determined to be frameshift mutagens, which require metabolic activation (cf. Chapter III-2.3.2). Another striking observation was that in vitro EROD induction, in vivo embryotoxic/teratogenic effects and the content of PAHs in the sediments could be significantly correlated to each other (cf. Chapter III-2.3.5).

This strongly corroborates the suggestion that PAHs are among the responsible compounds that triggered the CYP1A induction and phenotypes of D. rerio. Although a relationship between the total concentrations of PAHs in this study, which induce mutagenicity in the Ames assay, to the observed mutagenic effects in the individual samples could not be found, hints the
required attribute “metabolic activation” also to PAHs. The missing relationship may be explained by the nature of the mutagenic effect, which is based on probability rather than a directly receptor-mediated induction (cf. Chapter III-2.3.5). Overall, the particle-bound pollutants showed only a low bioavailability, which on the one hand further hints at a lipophilic character of the causative agents, but also means that organisms are less exposed to them until remobilized (cf. Chapter III-2.3.4 and Chapter III-2.3.6).

1.6.2 Non-target compounds

Aside the detected compounds also an involvement of non-target compounds, likely derivatives of the measured PAHs, e.g., nitro-PAHs and heterocyclic PAHs (NSO-Het), has been proposed. Specifically because these compound classes exhibit effect characteristics, which correspond to the observed effects in vitro, in vivo and in situ of this study (cf. Chapter III-2.5).

Thus, the focus should be extended further to non-target pollutants to identify more causative agents, including emerging pollutants. In order to do so, bioassay-guided effect directed analysis has been proven to be a valuable tool (Brack & Schirmer 2003, Brack et al. 2005, Hecker & Hollert 2009).

It has also to be considered that only organic pollutants were in the scope of this study. Inorganic contaminants, like heavy metals or high nitrogen levels due to excessive regional fertilization, as well as other biotic and abiotic factors, like pathogens or oxygen depletion due to eutrophication by repeatedly occurring algal blooms, may also play their part as detrimental factors. A complex variety of pathways exist that influence the reservoirs biota.

1.7 In situ effect assessment and contamination levels in aquatic organisms

1.7.1 In situ contamination levels

The reviewed articles in Chapter III-1.4 chiefly focused on perfluorinated compounds (mainly PFOS) and PBDEs in fish (mainly carp and catfish), as well as organochlorine pollutants (mainly HCHs and DDTs) in mollusks and crabs. Yet there is only little knowledge available about the predominant priority pollutants that could be measured along the Yangtze River, like PCBs and PAHs, present in and their influence on aquatic organisms of this area. They have been demonstrated to potentially induce toxicopathic hepatic lesions in fish after long-term low exposure or short-term high exposure (Myers et al. 1998, Ortiz-Delgado et al. 2007).
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The presence of PAH metabolites in bile of the benthic darkbarbel catfish *Pelteobagrus vachellii* from the TGR was verified in this study. Comparing the measured concentrations of 1-OHPyr to threshold values for cod (*Gadus morhua*) and turbot (*Scophthalmus maximus*) set by the ICES (2012), it could be seen that the majority of the samples exceeded these values. This indicates that the PAH contamination in fish from the TGR is of significance and may rise concern for the fish health in some cases. However, the cited threshold values provide only a rough guidance because they have been calculated for marine fish and due to the low number of available replicates these results should only be considered as indications (cf. Chapter III-2.4.2).

1.7.2 *In situ* toxicological endpoints

Concerning measured toxicological endpoints hardly any were examined *in situ* along the River, except genotoxicity by the formation of micronuclei in erythrocytes of carps and catfish from the Yangtze River’s Lower Reaches (cf. Chapter III-1.4.2) (Chen et al. 2002c, Li & Shen 2010). A significant formation of micronuclei was also found in *P. vachellii* from Chongqing (CNG 2013: 2.250 ± 2.073 %), and Kaixian (HF 2012: 0.400 ± 0.175 %; HF 2013: 1.450 ± 2.040 %) from the TGR in this study (cf. Chapter III-2.4.1). They are comparable to those of common carp *C. carpio* (3.56 to 4.50 %), grass carp *C. idellus* (3.55 %) and Chinese longsnout catfish *L. longirostris* (4.68 %) detected by Chen et al. (2002c) in the Lower Yangtze Reaches. The DNA damages prove environmentally relevant concentrations of pollutants in the water and demonstrate the relevance of genotoxicity as an important mode of action in the Yangtze River’s fish. Chen et al. (2002c) pointed out that the observed formation of micronuclei might be associated with accumulated volatile phenol and petroleum hydrocarbons in the fish from the Lower Reaches (inter alia in *P. vachellii*, which were not tested for micronucleus formation). A similar association, that PAHs and their derivatives are the causative agents, was made for the catfish sampled in this study (cf. Chapter III-1.4.2 and Chapter III-2.4.1).

The control groups from the Lower Reaches had a micronucleus frequency of 0.82 % and 0.093 % for the benthopelagic carp species *C. carpio* and *C. auratus*, respectively. In comparison, the control group of the benthic darkbarbel catfish *P. vachellii* had a micronucleus frequency of 0.200 ± 0.158 % (BJX 2012). This emphasizes the individuality of baseline micronucleus formation between several species. Therefore, it is crucial to determine a respective reference value for each species for a sound interpretation of the results. To the authors knowledge, this study provides the first reference value for micronucleus frequency in
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*P. vachellii*, which is one of the species with major economic importance in China (Ministry of Environmental Protection - China 2007) *(cf. Chapter III-1.4.2 and Chapter III-2.4.1).*

In addition to genotoxicity, the *in situ* investigations suggested a rather poor condition of *P. vachellii*, with histopathological alterations in liver and excretory kidney *(Chapter III-2.4.3 and Chapter III-2.4.5).* Moreover, fish from Chongqing city exhibited significant hepatic EROD induction and obvious parasitic infestations *(Chapter III-2.4.4 and Chapter III-2.4.5).*

PAHs have been identified as key pollutants all along the Yangtze River. As described in Chapter III-3, it has been demonstrated in rainbow trout *O. mykiss* that PAHs, like benzo[a]pyrene, are not just distributed to the liver, but also in similar levels to the immune organs head kidney and spleen. They further possess, although significantly different in their intensity (liver > head kidney > spleen), the capacity to metabolize benzo[a]pyrene to the potentially immunotoxic main metabolite BaP-7,8-dihydrodiol. This means in consequence, that immunotoxicity has to be considered as a relevant mode-of-action in the Yangtze River.

### 1.8 Bioaccumulation and risk of secondary intoxication

As wild fish and other aquatic organisms still remain an important dietary source in China (Su et al. 2012), bioaccumulation of pollutants in the aquatic organisms has to be considered. The accumulation poses a risk to the organisms on the one hand and on further consumers on the other, as shown by Scholz-Starke et al. (2013). Particularly, as most of the dominating substance classes identified in the Yangtze River are lipophilic and share the critical property persistence. The sustenance with polluted food, e.g., fish, muscles and crabs, might lead to secondary intoxication. This can either result in acute toxicity or to effects like higher risk for cancerogenicity after chronic exposure to pollutant levels that exceed the acceptable daily intake.

With respect to uptake and bioaccumulation of organic compounds the reviewed articles suggested that a basic risk of bioaccumulation exists, which could be demonstrated by Shao et al. (2005) for nonylphenol and nonylphenol ethoxylates in fish from Jialing River and Yangtze River at Chongqing section prior to the TGR’s impoundment, as well as by Ma et al. (2008) for OCP accumulation in shellfish in the Yangtze Estuary *(cf. Chapter III-1.4.1).* In case of the TGR after impoundment, on the one hand the pesticides Picoloram and Clopyralid measured by Wolf et al. (2013a) are not bioaccumulative *(Buchwalater et al. 2002a,b).* On the other hand, the determined PAHs in this study generally persist in the environment with half-lives from days to years, depending on the individual structure and processes like photo- and biodegradation.
(Shuttleworth & Cerniglia 1995, Kanaly & Harayama 2000, Fasnacht & Blough 2002). It is believed that aquatic organisms mainly take up the more water-soluble PAHs through ventilated water, and that the primary route of uptake of more hydrophobic PAHs is through ingestion of food or sediment (Meador et al. 1995, Hudjetz et al. 2014). Thus, they can accumulate in organisms without the right metabolic detoxification systems due to their lipophilicity, and consequently biomagnify through trophic transfer (Coates et al. 1997, Kanaly & Harayama 2000). However, they are easily biodegradable in organisms with the right metabolic mechanisms (cf. Chapter III-3) (Van der Oost et al. 2003, Möller et al. 2014), unfolding their toxic potentials through biotransformation to reactive intermediates (WHO 2010). The uptake of PAHs into the catfish sampled in this study could be shown by the detection of biliary PAH metabolites, as well as hepatic metabolic activation and genotoxic impacts that likely have been induced by them (cf. Chapter III-2.4.1 and Chapter III-2.4.2).

However, despite the observed accumulation of organic pollutants in organisms the reviewed articles revealed only a low risk of secondary intoxication with organic pollutants by consuming aquatic organisms from the Yangtze River. This stands in agreement with the uptake of PAHs by *P. vachellii* in this study, which are likely metabolized and excreted before they can biomagnify in the food chain. They rather unfold their toxic properties during the biotransformation process in the respective organs of the fish, like liver, head kidney and spleen.

To gain a comprehensive view on the potential risk it would yet be necessary to extend the variety of investigated organic pollutants in biota to other important bioaccumulative toxicants, e.g., PCBs. They have been considered only in two studies, though they could be widely measured in water and sediment along the river. Moreover, besides organic components also heavy metals are known to accumulate in biota and therefore need to be integrated in an overall risk assessment as well (cf. Chapter III-1.4.3).

To summarize, prior to a risk of secondary intoxication for humans the detected organic contaminants pose a higher risk for adverse effects in the aquatic organisms themselves.

### 1.9 Role of tributaries

The review of the research done along the Yangtze River in the past two decades showed that many tributaries presented higher pollution levels and toxicity than the mainstream. Particularly at some economic centers such as Chongqing (Jialing River) and Wuhan (Han River) (cf. Chapter III-1.5). Yet, the sediment analysis in scope of this study drew a different picture of the situation for the TGR section after impoundment. Apparently, samples from the mainstream
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exhibited higher levels of PAHs than the tributaries with the general pattern: upstream > downstream > tributary (cf. Chapter 2.2.1). With regard to the registered effects, no distinctive pattern could be identified for mutagenicity (cf. Chapter III-2.3.2), while the pattern of EROD induction and overall effects in the FET displayed the general pattern: upstream > tributary > downstream (cf. Chapter III-2.3.3 and cf. Chapter III-2.3.4). The discrepancy between these observations, that the tributaries have higher induction potentials than the downstream, can most likely be attributed to a number of interactions between the mixtures of analyzed and non-target compounds, that could not be registered within the scope of this study. Generally, in average only about half of the measured PAHs could explain the detected mutagenicity (Campaign 2011: 55 ± 9%; Campaign 2013: 48 ± 8% at Chongqing; 42 ± 7% at Kaixian), and even less the in vitro EROD induction (Campaign 2011: 43 ± 9%; Campaign 2013: 34 ± 7% at Chongqing; 32 ± 11% at Kaixian), assuming only additive effects (cf. Chapter III-2.3.6). The finding that the TGR tributaries were mainly affected by petrogenic sources (cf. Chapter III-2.2.5 and Chapter III-1.3), may hint to an explanation and underlines that not just the quantity, but also the quality of the pollutants matter.

In Chapter III-1 the lower levels of contamination in the Yangtze River’s mainstream, compared to its tributaries, were referred to the vast amounts of water carried by it. The mainstream incorporates also less polluted tributaries and thereby dilutes the input and toxicity from higher polluted sources. This demonstrates the need to also monitor and initiate countermeasures at tributaries of the entire Yangtze River network, which are home to numerous people and habitat of a large variety of biota.

With the impoundment of the TGR section several hydrological conditions changed, which also altered the distribution and occurrence of pollutants in this area. The hydrological conditions in the Yangtze River in general and the TGR in particular are described in the following chapter.

1.10 Influence of hydrological conditions on pollution

1.10.1 Yangtze River

As outlined in Chapter III-1.5, concerning the question, if there is a solution of the pollution problem by dilution, there was a consent with the findings of Müller et al. (2008) that the Yangtze River’s immense amounts of water and sediment indeed reduce the ecotoxicological risk growing from pollution all along the river, but do not eliminate it.
As further described in Chapter III-1.5, several studies observed that the water level had an influence on the concentrations and effects of pollutants. Concentrations were generally higher during low water period in the dry season in winter/spring than during the rainy season in summer. This observation has been attributed to different hydrologic factors: on the one hand to the difference in amounts of discharged water and particulate matter, causing a greater or lesser dilution, and the reduced velocity during low water level on the other hand. The lower flow rate causes an enrichment of pollutants in the water as well as higher sedimentation rates, which lead to the accumulation of particle bound pollutants in sediments.

1.10.2 Three Gorges Reservoir

Due to the inverted water level situation in the TGR since impoundment, with high water level in winter and low water level in summer, it was postulated that the changes in hydrologic conditions manifest as changes in the contamination status, with greater pollutant and effect levels during rainy season than during dry season. Hydrophilic compounds were considered to be carried with the discharged water past the dam, but hydrophobic substances will most likely remain in the sediments of the reservoir. It has also to be kept in mind that the elevated precipitation in summer is associated with an increased amount of air-borne particles washed out from the air as well as a stronger runoff from fields, carrying pollutants into the TGR, which may also be trapped there and subsequently accumulate in this region. To verify this assumption it was recommended to continuously monitor these changes (cf. Chapter III-1.5).

The postulation corresponded to the analysis of PAH contamination in the TGR’s sediment, as described in Chapter III-2.2.1. It showed that the temporal variation of PAH concentrations between the campaigns September 2011 and May 2013 were generally higher in September 2011. These observations stand in agreement with Wang et al. (2013), who referred the temporal differences in pollution to the drawdown period of the TGR (around May), where water quality is better than during the other seasons.

1.10.3 Yangtze Estuary

The importance to look at rivers not in independent segments, but as one unit can be illustrated at the example of the Yangtze Estuary. Particulate-bound pollution, carried away from upstream sources, can manifest in the downstream course and influence distant areas. The intrusion of saltwater at the river’s mouth also causes a reduction in flow rate, which elevates the sedimentation rate and leads to an accumulation of particle bound pollutants. In addition, the increase in salinity can cause a shift in the pollutants affinity from water to sediment, as shown
for PFOS (Pan & You 2010). The consequence is that hydrophobic organic pollutants are trapped in the estuary. With respect to the high persistence of the detected compounds along the river, like PCBs, OCPs and some resistant PAHs, in addition to the enduring production of emerging pollutants, it is to be expected that the Yangtze Estuary will continue to suffer the pollution discharge of the river and that the contamination in this area will still increase in the future (cf. Chapter III-1.5).

1.10.4 Mass balance approach

As described in Chapter III-1.5, Europe faced and still faces comparable issues as the Yangtze River basin. The Rhine, which is one of the economically, socially and environmentally most important rivers in Europe, and also an intensely examined and monitored water body, can serve as an example for future approaches concerning the Yangtze River. Though concentrations of organic pollutants may be lower in the Yangtze River than in the Rhine River, due to a comparably higher mass transport of water and particulate matter, it still results in comparably larger amounts of organic pollutants that end up in the Yangtze River’s receiving water body. The Yangtze River’s mean water discharge (30,200 m³/s) (National conditions - China 2003, Li et al. 2012a) is about fourteen times greater compared to the Rhine River (2,200 m³/s) (Huisman et al. 2000). This implicates that at same contamination levels in both rivers still the fourteen fold amount of toxic substances would enter the East China Sea. Therefore the impact of the Yangtze River on the East China Sea needs to be taken into account.

The same dilution effect is true for the TGR section. Over the past hundred years, the annual water discharge of the upper Yangtze at Yichang – at the TGD’s location near Sandouping – has varied between 5,000 m³/s in the dry winter and 40,000 m³/s in the rainy summer months (cf. Chapter I-3). And since the operation of the dam annually 151-172 Mt of sediment have been trapped in the TGR (2003-2008) (Yang et al. 2007, Hu et al. 2009a), which accounts for about 60-68% of the sediment entering the TGR from upstream. This also has serious consequences for the regions downstream of the dam – especially the Yangtze Estuary –, which rely on continuous sediment supply (Yang et al. 2007, Xu & Milliman 2009) (cf. Chapter I-4). Comparing the immense sediment influx to the PAH analysis of this study a deposition of 216-636 kg PAH/day (0.2-0.6 mg PAH/m³/day) in the TGR area is suggested, indicating an ecotoxicological risk. These numbers are in agreement with the mass balance estimation of Müller et al. (2008) that about 500 to 3,500 kg of phenols, chlorinated compounds, aromatic hydrocarbons and PAHs are discharged by the Yangtze River per day (cf. Chapter 2.2.1). A large amount of the deposited PAHs could be referred to air pollution (cf. Chapter IV-1.3).
And as PAHs pass several exposition pathways – air and water – they pose a risk to humans and wildlife already before they are deposited.

Thus, in Chapter III-2.7 and Chapter IV-1.3.4 it was concluded that air pollution, which plays a considerable role in China (Zhang & Tao 2009, Zhang et al. 2009b), also affects the quality of its water bodies. Zhang and Tao (2009) estimated an annual atmospheric emission of 114,000 t PAH$_{2,16}$ for China in 2004 – accounting for 22% of the global emission, followed by India (17%) and the United States (6%).

Compared to the external sources, like air pollution, it was suggested that the risk of internal pollution emission from the TGR’s submerged sites plays only a minor role, due to the deposition of vast amounts of sediment in the reservoir and the dilution effect.

1.11 Socio-economic costs of pollution in China

1.11.1 China’s economic growth

China’s enormous economic growth spurred its giant leap from a developing country to the brink of a developed country in the past decades. With an annual increase in GDP of 8-9 % about 400 million people were lifted out of critical poverty, and China climbed from rank 108th to rank 72nd on the World Development Index between 1979 and 2005 (World Bank 2007). With a GDP growth of 7.7 in 2013 and an expected GDP increase of 6.9 in 2017 (World Bank 2014), a high pressure on the environment and its resources remain to exist. However, there are serious concerns about the long-term sustainability and hidden costs of growth, from which numerous are associated with impacts of air and water pollution. This growth already came with a high toll in environmental and public health – in a country that considers health as one of the highest values (cf. Chapter I-1) (World Bank 2007, Blacksmith Institute et al. 2015).

1.11.2 The socio-economic impact of air, water and soil pollution

Out of the world’s 20 cities with the worst air pollution 16 can be found in China, primarily due to heavy industry, metal smelters and coal-fired power plants. The Chinese government estimates that air pollution alone is killing about 300,000 people annually, with costs of billions of dollars in lost productivity and healthcare expenses every year (Blacksmith Institute et al. 2015).

In addition to air pollution comes the contamination of soil and water, primarily from mining, smelting and industrial chemical manufacturing (Blacksmith Institute et al. 2015). Heavy
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metals pose a serious threat to China’s biodiversity, public health and agriculture. More than 10% of the cultivated land was announced to be polluted with heavy metals, causing a loss of up to 12 million tons of food each year (Blacksmith Institute et al. 2015). Bao et al. (2012) described the levels of POPs in China’s drinking water sources and coastal waters to be generally at the high end of the global range, with several indications of human health risks. Particularly the booming eastern regions, where affected while data on the western regions was scarce (Bao et al. 2012).

The World Bank (2007) reported that between 2001 and 2005 in average about 54% of the seven main Chinese rivers contained water, which was considered not safe for human consumption – representing an about 12% increase since the early 1990s. The rivers with the highest contamination status could be found in the northeast territories with high population density. Overall, the trends in surface water quality from 2000 to 2005 suggested that the situation becomes worse in the northern main river systems, while those in the South slightly improve. This corresponds to the general observation made on the Yangtze River in the scope of this study that the water quality generally met the Chinese and European standards (Ministry of Environmental Protection - China 2002, EWFD Directive 2008/105/EC) (cf. Chapter III-1.5, Chapter III-2.7 and Chapter IV-1.4).

Further, the World Bank (2007) reported that fishery losses due to acute water pollution accidents in 2003 amounted to over 4.3 billion Chinese Renminbi Yuan (RMB) (about 520 million US dollar) – including 713 million RMB in direct economic losses and over 3.6 million RMB in indirect losses (Ministry of Agriculture & SEPA - China 2003, World Bank 2007). However, these numbers are considered to greatly underestimate the real economic cost of fishery loss caused by pollution. Chronic pollution costs are likely to exceed the acute, and the methodology to calculate the indirect costs – the rule that those cannot be higher than triple the direct – may be an additional underestimation of the true costs of pollution (World Bank 2007).

Aside from the direct threat on health from exposure to contamination, water pollution also has indirect consequences, like water scarcity. While contaminated surface water is avoided, people reach out for alternatives causing a serious depletion of ground water (World Bank 2007). This means that paired with the climate change additional pressure is put on the water resources. Another consequence is the destruction of harvest and contamination of food. In some areas farmers are forced to irrigate their crops with wastewater, causing serious damage on their harvest. It has also been reported that they often do not dare to eat the crops themselves, using
scarce clean water for their own purpose, and instead export the goods (World Bank 2007, NY Times 2013).

Overall, air and water pollution particularly affected the poor, and air pollution weighed more problematic than water pollution in China (Eldis 2007, World Bank 2007). One reason are the different uptake pathways, which are generally better in the lung, compared to skin and indigestive tract. On the other hand, water pollution is more difficult to grasp, which may underestimate the real risk. People are exposed frequently to air, but not frequently to water. The infrequent exposure to water impedes the relationships with health incidents (World Bank 2007).

Air and water pollution were considered to cause economic losses equal to 5.8 % of China’s GDP in the first years of the beginning new century – health impacts accounting for 4.3 % and non-health impacts for 1.5 % (Eldis 2007, World Bank 2007). Other sources claimed that the “green GDP”, which is thought to include environmental degradation and the cost of natural resources, was underestimated by the Chinese government, who announced that environmental pollution caused economic losses of 3.1 % of its GDP in 2004 – equally to about 64 billion US dollar. The real costs for the Chinese economy rather had to be figured between 8 to 12 % annually – equally to 168 to 252 billion US dollar (PBS 2006). The South China Morning Post (2013) spoke of 3.5 % in 2010 – equally to 1.54 trillion RMB or 227 billion US dollar (World Bank 2015) –, citing a report published by the “Chinese Academy of Environmental Planning”, which belongs to the Ministry of Environmental Protection.

Although all these numbers differ, particularly depending on the incorporated data and calculation methodology, they all have in common that they drastically reduce the actual economic growth.

In 2010, the costs of pollution grew faster than the GDP, and the report by the Chinese Academy of Environmental Planning stated that “the existing accounting system fails to reflect the true cost of resources consumption and environmental degradation, as a result the country's economic achievement has been over exaggerated” (South China Morning Post 2013). This means, that the growth is actually much weaker, if not completely nullified, and that the environmental expenses may even lash back in the following decades.
1.11.3 Countermeasures

The Chinese government has understood the economic impact on economy and health, and initiated several countermeasures – with diverging success (He et al. 2012). In order to tackle the root causes of air pollution, measures have been initiated to cap regional coal consumption, reduce coal power plant emissions, enforce cleaner fuel standards, close inefficient coal-fired industrial boilers and restrict constructions of power plants and other energy intensive industries near residential areas. Further, the population has been encouraged to switch to electric vehicles and been better informed by policy changes, which include the publication of air quality information (Blacksmith Institute et al. 2015). Further, the latest Five-Year-Plan aimed at a reduction of 15% of heavy metal emission below 2007 levels in “priority areas” and a limitation of emissions to 2007 levels in “non-priority areas”. In addition, a comprehensive system for the control of heavy metal pollution was implemented (Blacksmith Institute et al. 2015). Though China has built the organic pollutants management framework in the past two decades, it still lagged behind developed countries a couple of years ago, with regard to the control of certain POPs and emerging pollutants in water and sediments (cf. Chapter III-1.2.6) (Wang et al. 2005). The Chinese Academy of Environmental Planning’s report admitted that government campaigns targeting major air and water pollutants since 2006 did not manage to reverse the trend of rising environmental costs (South China Morning Post 2013). In response to the amount of 1,000 Mt urban and industrial wastewater, which were discharged into the TGR area annually during the impoundment period, the local governments adopted policies on emission reduction for the industry. They also improved the domestic wastewater treatment capacities from annually 515 Mt in 56 facilities in 2008, to 590 Mt in 71 facilities in 2010. However, both times domestic sewage contributed for about 98%, meaning that still 88 Mt domestic and 548 Mt industrial sewage in 2008, as well as 37 Mt and 307 Mt, respectively, in 2010 were discharged untreated (cf. Chapter I-4) (Ministry of Environmental Protection - China 2006-2012, Wang et al. 2013). The construction of the Three Gorges Dam can also be understood as a countermeasure to tackle the growing need for energy, as a “cleaner” alternative to the combustion of fossil fuels, which are the main contributors to the country’s air pollution problem. However, this caused environmental impacts in its turn, like the flooding of fertile shorelands and increasing landslides, as well as a change of the hydrological conditions, habitat fragmentation and shrinkage, which are considered to cause a shift in age and species composition, in addition to a general fish decline (cf. Chapter I-4 and Chapter III-2.7).
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Just at the beginning of this year China introduced a new improved Environmental Protection Law, which is considered to be far more stringent and includes an elemental aspect in environmental protection – provisions for public education. This underlines the determination of the Chinese government to tackle and eventually reverse the impacts of pollution on economy and health (Blacksmith Institute et al. 2015, South China Morning Post 2015).

A core requirement of implementing, enforcing and control environmental protection measures is knowledge. Data on the contamination status of air, water and soil is crucial for decision-making, in order to take the appropriate actions. For example, the problem to actually grasp the real cost of pollution was referred to a lack of data, which emphasizes the need for nationwide long-term monitoring programs (PBS 2006, World Bank 2007, Bao et al. 2012, South China Morning Post 2013).

The work performed in scope of this study shall support the decision-making process and supply suggestions for monitoring methods, in order to get a comprehensive view on the TGR, the Yangtze River and, taking them as examples, other Chinese water bodies (cf. Chapter I-5 and Chapter III-2.7).
2 Recommendations

2.1 General recommendations

- The Yangtze River should be seen in context of the River Continuum Concept (Vannote et al. 1980) and the Catchment-Coastal Sea Continuum (cf. SedNet 2004, Salomons et al. 2005), approaches that describe and evaluate a river as an interlinked unit, considering it as an open and holistic ecosystem. The River Continuum Concept describes the river from its source to its estuary and integrates biotic parameters, physical and hydrological factors, energy and nutrient input as well as output. Moreover, the Catchment-Coastal Sea Continuum further emphasizes the impact of the river on the receiving sea and its coast, which is of particular importance for the Yangtze River as demonstrated by the mass balance calculations in Chapter IV-1.10.4. The underlying concepts have been implemented (in its limits) for European river systems and coastal waters within the European Union Water Framework Directive (EWFD Directive 2000/60/EC) (cf. Chapter III-1.5).

- Long-term monitoring programs are required to surveil the quality of air, water and soil, in order to supply reliable information for decision-making processes and environmental management (cf. Chapter IV-1.11).

2.2 Monitoring Methodology

- In order to meet the complex situation in the field a holistic assessment in environmental monitoring is required (cf. Chapter I-6) (Heugens et al. 2001, SedNet 2004, Hollert et al. 2007, Brils 2008, Hollert et al. 2009, Malaj et al. 2014, Wernersson et al. 2015). This study has proven the suitability of the triad approach with additional lines of evidence in the Yangtze TGR area, combining chemical analysis with in vitro, in vivo and in situ approaches (Chapman 1990, Chapman & Hollert 2006). In vitro and in vivo bioassays allowed for a fast screening of environmental samples and helped to identify relevant modes of action. In situ approaches included higher organismic levels with a greater ecological relevance and the possibility to prove the relevance of lab-based assays for the situation in the field. In addition to that, chemical analysis helped to identify possible causative agents and individual key pollutants of the area. This allowed to reveal the respective emission sources for the implementation of countermeasures (cf. Chapter I-7 and Chapter III-2.7).
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- The chemical and ecotoxicological approaches should be supplemented with hydraulic approaches, e.g., to estimate the risk growing from remobilization of pollutants during flood events (Wölz et al. 2009, Brinkmann et al. 2010). This is particularly important for the Yangtze River, due to the high mass transport of water and sediment, as well as the frequently occurring flood events in the river (cf. Chapter I-2 and Chapter I-6).

- The role of the tributaries, as potential pollution sources, should be included in a holistic assessment of the water bodies (cf. Chapter IV-1.9).

- The monitoring methodology should be based on mixture samples to cover intrinsic heterogeneity of each sampling location – water and sediment (cf. Chapter II-2.2).

- The number of replicates of sampled biota, e.g., fish samples, should be ten at minimum. As results from biota samples typically exhibit high oscillations, higher numbers of replicates provide more reliable results.

- Bioassay-directed analysis is recommended as an additional tool to identify relevant toxic components and non-target pollutants (Brack & Schirmer 2003, Brack et al. 2005, Hecker & Hollert 2009).

- In order to seize the entire spectrum of environmental impacts, other factors have to be considered aside organic pollution. Inorganic contaminants (like heavy metals or high nitrogen levels, due to excessive regional fertilization), as well as other biotic and abiotic factors (like pathogens or oxygen depletion, due to eutrophication by repeatedly occurring algal blooms) also play their part as detrimental factors. In addition, land use changes and the construction of dams, which influence the river’s ecosystem and hydrological conditions, have to be kept in mind and included into an overall assessment (cf. Chapter III-1.5 and Chapter III-2.7).

2.3 Chemical compounds

- Measuring the three major compound classes PAHs, PCBs and OCPs should be part of the standard monitoring in Chinese rivers (cf. Chapter III-1.2.6).

- As urbanization effects on the environment can be recorded by PAHs in sediment (Hong et al. 2012), are PAHs hereby suggested as suitable marker compounds or indicators for monitoring of the sediment pollution status. This is in accordance with Wang et al. (2013), who stated the same for water (cf. Chapter III-2.7).

- More research on emerging pollutants in the Yangtze River is required (cf. Chapter III-1.2.6).
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- To estimate the entire risk growing from bioaccumulation to the organisms and the consumers it is recommended to intensify the research on especially hazardous and well known bioaccumulative contaminants. For example, the in water and sediment ubiquitously detected PCBs, as well as heavy metals (cf. Chapter III-1.5).

2.4 Biological effects

- Mutagenicity, genotoxicity, AhR-mediated activity, embryotoxicity/teratogenicity, immunotoxicity and endocrine activity should be considered as relevant modes-of actions in the Yangtze River (cf. Chapter III-1.5, Chapter III-2.7 and Chapter-IV 1.7).

- The in vitro EROD induction assay and Ames fluctuation assay, as well as the in vivo Fish Embryo Toxicity Test and Sediment Contact Assay, in addition to the in situ EROD/GST induction assays and micronucleus assay approved as comparably simple, cost-effective and reliable methods. Thus, they are highly suitable for knowledge transfer in general. The histopathological evaluation of the fish offers valuable information on the organ level and is simple to prepare, but requires long-term training and experience for a qualitatively appropriate evaluation (cf. Chapter III-2.7).

- In vitro/in vivo exposure scenarios should be performed with native samples and extracts to register the effects of the total extractable content and the bioavailable fractions of contaminants (cf. Chapter III-2.7).

- The benthic darkbarbel catfish Pelteobagrus vachellii has been proven to be a suitable monitoring fish species and can be recommended for further monitoring approaches. The positive aspects were its demersal lifestyle, low to medium migration – thus being suitable to indicate local contamination over longer periods of time –, its wide distribution along the Yangtze River and its major economic importance (cf. Chapter II-2.2). In combination with water monitoring rather benthopelagic and pelagic species are recommended, like the also economically relevant grass carp Ctenopharyngodon idellus or common carp Cyprinus carpio, respectively.

- Effects of chronic exposure to contaminants need to be taken into account, particularly as mutagenic and endocrine activities of pollutants in low concentrations cannot directly be set equal to low risk. For mutagenicity, due to the mutagenic mode of action threshold levels are under discussion. And with regard to endocrine activity, marginal concentrations even below the limit of detection can lead to severe impacts on the population level. For example, as shown for 17α-ethinyl estradiol almost causing the
extinction of an entire fish population in a Canadian lake (Kidd et al. 2007) (cf. Chapter III-1.3.2 and Chapter III-2.7).

2.5 Countermeasures

- Countermeasures to reduce the emission of PAHs into the environment are necessary, e.g., catalysts for cars, pollutant filter for factories and chiefly alternatives to fossil energy sources (cf. Chapter III-1.5).
- Attention should be paid to the release of PCBs, as well as PBDEs, into the environment from improper importation and disposal of electronic waste along the river (cf. Chapter III-1.5).
- The usage of lindane and dicofol in some agricultural areas of the Middle and Lower Yangtze Reaches should be heeded as new input sources of DDTs and HCHs (cf. Chapter III-1.5).
- Toxicity reduction evaluation should be applied to areas with elevated toxicity levels, like Chongqing, Kaixian, Wuhan and the Yangtze Estuary (cf. Chapter III-1.5 and Chapter III-2.7).
- Advanced methods of wastewater treatment, like a substitution for the chlorination of polluted source water by less harmful water treatment methods in the preparation of drinking water, and integrated strategies to minimize the impact of point and non-point sources should be further developed. For example, by transfer of knowledge from national research programs and applications (Huckele & Track 2013, Triebskorn et al. 2013) to bilateral and international joint projects in China (e.g. Bergmann et al. 2011 and Clean Water Initiative, China) (cf. Chapter III-1.5).
- Prevention, including both direct and diffuse pollution control, should therefore be the first choice, remediation the second. As demanded by the EWFD the precautionary principle in combination with continuous monitoring of the “whole water body”, including water, sediment and biota, is strongly recommended (EWFD Directive 2000/60/EC, SedNet 2004, Hollert et al. 2007, Brils 2008, EWFD Directive 2008/105/EC) (cf. Chapter I-6 and Chapter III-1.5).
Chapter V

Overall Conclusion
Overall Conclusion

The initial question, “Is the Yangtze Three Gorges Reservoir not just dammed, but also damned?” could be answered based on the ecotoxicological research encompassed by the scope of this study. Despite the consideration of the environmental challenges the TGR faced since its impoundment, such as submersion of abandoned contaminated sites and rising ship traffic, in combination with a progressive urbanization and industrialization of the area, the environmental impacts of organic pollution between September 2011 and May 2013 were less distinct than expected. Generally, the detectable organic pollution situation in the TGR was comparable or even lower than in national and international comparison. Overall, the Yangtze River’s water quality mainly met national and international quality criteria. In addition, most parts of the TGR did not exhibit significant ecotoxicological effects on the organism level, and if, only to a minor degree. This could also be registered in the in vivo Sediment Contact Assay with Danio rerio. Thus, with regard to the reported fish decline in the Yangtze River, it was deduced that organic pollution should be taken into account, but at least the acute toxicity is subordinate to resources overexploitation and habitat destruction.

In contrast to that, mutagenicity, AhR-mediated EROD induction and embryotoxic/teratogenic effects were widely detectable in the sediment extracts from most TGR sites. Therefore, it can be concluded that the causative agents and ecotoxicological potentials exist, but crucial factors, such as dilution, bioavailability of the compounds, exposition pathways, metabolism and excretion of the organisms, reduce the impact. This hidden potential shifts the necessity to understand remobilization processes into the foreground and demonstrates the importance of incorporating hydrological processes into environmental management. This is particularly true for the TGR and its special hydrological conditions: decreased flow velocity, inversed water level situation between dry and rainy seasons, or the impact of the mainstream on the tributaries by a flow direction reversal due to an increasing water level. The TGR is therefore well suited as an example for other dam and reservoir projects.

Apart from the generally less polluted state of the TGR area and the Yangtze River, they both exhibited some hot-spot sites with significant local ecotoxicological impacts. Wuhan and the Yangtze Estuary are particularly prominent examples, with Chongqing and Kaixian being of concern specifically in the TGR area. These sites require a toxicity-reduction evaluation and customized countermeasures, e.g., improved wastewater treatment in areas where sewage was a significant emission source.
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The relevance of the ecotoxicological endpoints mutagenicity, genotoxicity, AhR-mediated EROD activity, embryotoxicity/teratogenicity, as well as endocrine activity, could be demonstrated in the Yangtze River basin. Therefore, they should be incorporated in the monitoring programs of the TGR area and other Chinese water bodies. This is particular important as PAHs generally play a central role as key pollutants along the Yangtze River and the TGR in particular, which could be connected to the observed effects with a high probability. The capability of the immune organs in fish to metabolize PAHs to mutagenic intermediates further adds immunotoxicity to the relevant endpoints, and should be considered in environmental surveillance as well. Apart from that, as only a fraction of the effects could be explained by the analyzed compounds, it is necessary to identify additional organic non-target compounds. To this end, bioassay-guided effect directed analysis can be the beneficial method of choice (Brack 2003, Hecker & Hollert 2009). Additionally, it is necessary to incorporate inorganic contaminants, like nutrients and heavy metals, into the assessment.

Generally, based on the results from the biomarker investigations, as well as sediment and water analysis on the TGR and the Yangtze River, it was concluded that risk rather exists from chronic long-term low-dose exposure instead from high-dose acute toxicity; however, this should not be interpreted as an all-clear signal, in particular with respect to mutagenic, genotoxic and endocrine active compounds, as these pollutants can have severe consequences even in minor concentrations. As outlined in Chapter I-1, pollution is typically not immediately lethal. It rather acts as a risk factor and catalyst enhancing the rates of cardiovascular diseases and cancer, health impacts that can emerge from a misregulation of the AhR receptor and mutagenic/genotoxic effects, respectively; the relevance of both endpoints has been demonstrated in the TGR in scope of this study. Moreover, sublethal damage influences the overall fitness and health of organisms, thus affecting population health and economy.

Although the high dilution factor of the Yangtze River reduces the risk of acute pollution, it is not the solution of the pollution problem. Apart from the remaining chronic low-dose concentrations, the mass balance estimations in this study and of Müller et al. (2008) clearly demonstrate the enormous amounts of organic pollutants that are deposited in and carried down the river every day (cf. Chapter III-1.5 and Chapter III-2.7). Thus, the problem is not solved, only relocated.

One of the prior affected areas is the Yangtze Estuary. Several hydrological factors, particularly the intrusion of saltwater and increase of salinity, support the sedimentation rate of suspended particles carried down the river, and can increase the pollutants affinity from water to sediment,
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which then accumulate in the estuary. It is therefore crucial to conceive the Yangtze River in terms of the “River Continuum Concept” (Vannote et al. 1980) and the “Catchment-Coastal Sea Continuum” (cf. SedNet 2004, Salomons et al. 2005). Moreover, not only the rivers have to be understood as a unit, but also the interconnection of the water with the air and soil. The atmospheric input of pollutants, like PAHs, OCPs and PBDEs, could be outlined in this study; the runoff from land was described as an input source of OCPs, from agriculturally used areas, as well as PCBs, from improper disposal and leakage of PCB containing equipment. Both air and soil leave their footprint on the water bodies. In return, scenarios like the frequently occurring Yangtze River floods and the anthropogenically regulated water fluctuation of the TGR cause a relocation of dissolved and particle-bound pollutants on the riverbanks. These in their turn may be agriculturally used patches and fields, which deliver the contamination, in addition to contaminated fish and other aquatic organisms, to the table of human consumption. The risk of secondary intoxication from the consumption of fish and other organisms from the Yangtze River was described as low within this study, but it is essential to understand the interconnection and pathways the pollution may take and affect the life of people. Pathways, which allow the contamination of industrial centers to reach even remote places via atmospheric transport or the pollution emitted into the rivers in the inland to affect the estuaries far away. These pathways may even branch out transregionally as atmospheric transport and the course of rivers do not stop at man-made borders. Additionally, in a globalized world contaminated goods may travel even faster and farther than air and water could. "Our economy is global and so are the pollutants it generates." (Fuller 2015). These pathways circumvent national, local or even private protection measures and enter through the backdoor. This is, why we should not just care about our own doorstep, but also about our neighbors’.

Moreover, this not even considers the sociological and political implications growing from the depletion of substantial resources, like clean water, and the deterioration of an environment that prevents a provision with basic needs. These circumstances produce so called “environmental refugees” which have been defined by the United Nations Environment Program as “those people who have been forced to leave their traditional habitat, temporarily or permanently, because of a marked environmental disruption […] that jeopardized their existence and/or seriously affected the quality of their life.” (El-Hinnawi 1985). The estimated number of environmental refugees was at least 25 million in the mid-1990s, with more than 50 million in 2010, and over 200 million to be expected by 2050. This is a particular concern for Africa (south of the Sahara), China, South Asia and Central America, due to a lack of water, depletion of biodiversity, desertification and salination of irrigated land (Myers & Kent 1995, Myers 1997). In
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general, the main reasons are excessive regional contamination (“deposition”), slow environmental degradation (“degradation”) and natural catastrophes (“disaster”), which are connected with a wide-ranging destabilization of the social structure (“destabilization”) causing regional conflicts. Prominent examples, which have been influenced by environmental issues, are the “Zapatista Uprising” in Mexico, the “Tuareg Conflict” in Western Sahara Sahel, and even the “Rwandan Genocide” (Lume 1996, UNHCR 1997, Federal Agency for Civic Education - Germany 2002). The environmental refugees may even spread the disruption across national borders (Mathews 1989); however, the definition of migrants as environmental refugees and actual numbers are hard to grasp, because environmental deterioration is only one motive to migrate beside many more. The actual motives are commonly poverty, violence and war, but they often have their roots in ecological problems (Federal Agency for Civic Education - Germany 2002).

In China, particularly the north faces ecological problems, mainly water scarcity due to climate change, water pollution and consequently groundwater overexploitation. In general, the gradient of air and water pollution increases in China from west to east and south to north (World Bank 2007). The people that are forced to migrate and flee from environmental deterioration in rural areas, often end up in urban conurbations with even poorer environmental conditions (Chen et al. 2013) – thus exchanging the bad for the worse.

Referring to the western areas of China, including Tibet – the source of the Yangtze River –, the World Security Institute (Moore 2009) stated that “already poor and underdeveloped, these regions could experience rising inter-ethnic tension over the distribution of water or become a source of growing environmental out-migration as water-stressed inhabitants seek better opportunities elsewhere. Such migration has been documented in several parts of western China and identified by environmental security scholars as a key risk factor for environmentally-related conflict.” In addition to that, Morrison et al. (2009) stated by referring to Schneider and Pope (2008) that “studies by the Chinese Academy of Sciences and the Intergovernmental Panel on Climate Change (IPCC) suggest that increased industrial activities in the region [Western China], most notably logging, mining, and manufacturing, are severely affecting water quality, while climate change is hastening glacial melt and threatening water access and long-term supplies.” This points at a rising environmental concern in Western China and the Yangtze Upper Reaches, which were only scarcely investigated so far (cf. Chapter IV-1.1.1), but not only with consequences for China itself. Morrison et al. (2009) further outlined by referring to Schneider and Pope (2008) that “these concerns [water scarcity] significantly increase the risk of heightened political conflict and instability. China already considers water to be a crucial strategic asset. The depletion of its
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most importance source of water will only enflame conflict between itself and many of the region’s inhabitants. Furthermore, water scarcity will bring to the forefront looming concerns and potential conflict over water allocations between China and the governments of neighboring nations, such as India, Bangladesh, Vietnam, Cambodia, Thailand, Laos and Burma, which also rely heavily on water resources originating in Tibet.” The pressure on China for clean water supply is high, as it has about 7% of the worldwide water resources, but about 20% of its population with grave regional imbalances having four-fifths of the water resources in the south of the country (NY Times 2007).

Apart from these extreme consequences the foremost socio-economic impacts are still the costs on economy and health, which severely damages China’s development, with the danger of long-term consequences. However, the costs of pollution are not solely a Chinese problem; it damages the health and economy in all parts of the world, particularly affecting those countries with low environmental standards, which are typically developing countries, and thereby hindering their growth (Blacksmith Institute et al. 2015, WHO 2015c). Those who are hit hardest are the poor. In China, they can be particularly found in the rural and less industrialized parts (World Bank 2007).

The Washington post cited an OECD study with the words “When income inequality rises, economic growth falls”. Apparently, the wealth gap in OECD countries has never been wider in the past 30 years, and it has a serious impact on the countries’ economies. The given reason was that wealth gaps restrain the skill development of children, particularly those from families with poorer educational backgrounds. Failing to give poorer citizens access to high-quality and long-term education hurts the economy (Cingano 2014, Washington Post 2015). If this is true for developed western countries, what is the situation in developing and newly industrialized countries then? How can the gap of inequality be reduced if those poor don’t even have access to basic needs like clean water and food in a healthy environment? What happens when pollution affects the productivity of a population and prevents adults from working or children from going to school (OECD Observer 2007)?

China’s attempt to achieve a better economic equality throughout the country, initiated by programs like the “Rise of Central China” and the “Great Western Development Strategy”, therefore not only needs to strengthen the regional economies, but must also incorporate appropriate environmental protection and education measures. The Three Gorges Dam and its reservoir can be understood as a component of these strategies to tackle the economic inequality (cf. Chapter IV-1.11.3); however, the relocation of more than 1.2 million people due to the impoundment of the reservoir already showed the other side of the coin, in addition to the tens
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of thousands that will have to move again due to increasing risk from landslides (Reuters 2012). So, the TGD project also produced “environmental refugees”, with the risk for tensions and the entailing consequences. Therefore, the environmental problems that emerged from the implementation of the TGD project need to be carefully monitored and supervised, to prevent the costs from outweighing the benefits. Moreover, the sociological issues have to be taken seriously to prevent a conflict arising from a project that was intended to aid.

China is on a promising way to improve its environmental conditions, although it may still take a long time; however, the actions taken in the past two decades, reaching from import prohibition of electronic waste to stricter emission control standards and the new improved Environmental Protection Law 2015, demonstrate the determination of its government to approach the environmental issues and eventually reverse the impact on economy and health (Blacksmith Institute et al. 2015, South China Morning Post 2015).

The effort can be worth it. Literature reviews on how efficient different policy interventions to reduce water and air pollution are, suggest that those which improve the environmental quality are often cost-efficient, with the benefits outweighing the costs (OECD Observer 2007).

Nonetheless, Europe still has to learn and improve its environmental management as well. The EWFD’s main goal to achieve “a good ecological status” for all European ground and surface waters will not be achieved by 2015 for a significant proportion of water bodies (47%), although numbers improved since 2009 (57% in insufficient state) (Hollert et al. 2007, European Union 2012b). A recent intermediate report by the World Health Organization on the achievements scheduled at the 2010 Fifth Ministerial Conference on Environment and Health in Parma, Italy, stated that although substantial progress has been achieved in environment and health in the past decades, 25% of all diseases and deaths in Europe can still be attributed to environmental agents. The main causes of death are cardiovascular and respiratory diseases, type 2 diabetes and cancer – killing four out of five Europeans. With ageing populations and unhealthy lifestyles as considered main factors, new and stronger evidence refers these impacts on health to air pollution, chemical and physical agents as well as to climate change (WHO 2015c). Furthermore, inequality in the access to natural resources also persists in Europe. For example, Millenium Development Goal 7 on ensuring environmental sustainability in terms of water and sanitation was not accomplished in all European states (WHO 2015d). A WHO Europe survey from 2013 further pointed out the following shortcomings: (a) only half of the European countries report that they set up programs to reduce or eliminate chemical risks to children, (b) less than half of the states address priority carcinogens, mutagens, reproductive toxicants and
endocrine-disrupting chemicals, (c) less than half implemented a legal basis to prohibit the use of dangerous chemicals in products destined for children (WHO 2015c). Air pollution also damaged the European economy in 2010 with 1.6 trillion US dollar, due to 600,000 premature deaths and induced diseases. This is nearly equivalent to 10% of the whole European Union’s GDP in 2013 – in 10 out of 53 member states even 20% or more of the national GDP (WHO 2015a). So, although some member states already achieved better standards, Europe must continue to improve its environmental management.

From all this, it can be concluded that although the climate change was a major wake-up call, environmental issues are still often underrated. The main reason may be that their causes are often enough so difficult to grasp and surface only through the deterioration of health, poverty and sometimes even social conflicts. Another is surely the environmental education of the population, without their self-responsibility and awareness what consequences their actions may have the challenge is already lost. Therefore, a new perception, perspective and understanding of these problems is required, because environmental management and policy is not just a subsidiary task, it is a pivotal assignment – nationally and globally.

Above remediation of pollution should stand the precautionary principle, as demanded in the European Water Framework Directive (EWFD Directive 2000/60/EC), which in its turn can serve as a blueprint for future international environmental management strategies. Improving the environmental conditions upstream to avert environment-related issues in the first place can be far more effective than trying to treat the problems when they occur further downstream. For example, this can lead to significant cost savings for healthcare, or reduced surface water pollution to reduced energy expenditure and costs of drinking water treatment (OECD Observer 2007, World Bank 2007). This is in addition to the far more important improved life quality of the affected people.

However, a problem that could be identified within this study (cf. Chapter III-1) was that there is a considerable lack of ecotoxicological data on the Yangtze River, particularly concerning effect assessment and in situ data. Data deficiency was also criticized by the World Bank (2007) and the Blacksmith Institute et al. (2015), as being a major obstacle to adequately judge the environmental conditions and implications on health. For example, the World Bank (2007) declared in its report, that although there were many indications that surface and drinking water pollution contributed to serious health impacts in China, the lack of monitoring data on chemical and inorganic pollutants, in addition to exposure pathways made it difficult to assess the whole spectrum of health effects of water pollution.
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Therefore, national long-term monitoring programs are the necessary first steps in the sequence of understanding the problem and taking action. The proposed and successfully implemented concept of a holistic environmental assessment – applying the triad approach with additional lines of evidence – in scope of this study can be a valuable tool in this process.

The growing pollution of freshwater resources belongs to the central global environmental issues of the twenty first century (Schwarzenbach et al. 2006, Yang et al. 2012a). Hollert (2013) outlined that the lesson learned from the sino-german “Yangtze Project” is the requirement for long standing and sustainable cooperations to address complex and multidimensional environmental problems. These should be supported by bi- and multilateral, international research programs that address basic and applied research to develop conceptual strategies and technical solutions (Schaeffer et al. 2009).

With the intention of knowledge transfer, this study should represent a foundation for further monitoring programs of the TGR area, as well as the Yangtze River and other Chinese water bodies. Furthermore, as an evaluation of the reservoirs ecotoxicological status in the early days after its full impoundment it can serve as a reference for the future development of the TGRs environmental condition, parallel to the proceeding economic and demographic development of the TGR area. It should support the decision-making processes in order to initiate and enforce necessary countermeasures in time, to prevent environmental degradation in the long-term and sustain the unique Yangtze River ecosystem for the Chinese people.
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Supplement
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*Publications contributing to this thesis

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Scientific Contribution

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Zubrod J., Jevtic D., Melato A., Englert D., Weil M., Brockmeier E., Floehr T., Knezevic V., Agatz A. & Brinkmann M. (2013): News from the SETAC Europe Student Advisory Council (April 2013) - the 3rd Young Environmental Scientists (YES) meeting at the Jagiellonian University, Poland. Environmental Sciences Europe (25), 16.


Platform presentations


Poster presentations


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