Volume 251

Pim de Voogt *Editor*

Reviews of Environmental Contamination and Toxicology

Springer

Reviews of Environmental Contamination and Toxicology

VOLUME 251

More information about this series at http://www.springer.com/series/398

Reviews of Environmental Contamination and Toxicology

Editor
Pim de Voogt

Volume 251

Springer

Coordinating Board of Editors

Dr. Pim De Voogt, *Editor*
Reviews of Environmental Contamination and Toxicology

University of Amsterdam
Amsterdam, The Netherlands
E-mail: w.p.devoogt@uva.nl

Dr. Erin R. Bennett, *Editor*
Bulletin of Environmental Contamination and Toxicology

Great Lakes Institute for Environmental Research
University of Windsor
Windsor, Ontario, Canada
E-mail: ebennett@uwindsor.ca

Dr. Peter S. Ross, *Editor*
Archives of Environmental Contamination and Toxicology

Vancouver Aquarium Marine Science Center
Vancouver, BC, Canada
E-mail: peter.ross@vanaqua.org

ISSN 0179-5953 ISSN 2197-6554 (electronic)
Reviews of Environmental Contamination and Toxicology
ISBN 978-3-030-27148-0 ISBN 978-3-030-27149-7 (eBook)
https://doi.org/10.1007/978-3-030-27149-7

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife, and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the Earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol. 1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

The role of *Reviews* is to publish detailed scientific review articles on all aspects of environmental contamination and associated (eco)toxicological consequences. Such articles facilitate the often complex task of accessing and interpreting cogent scientific data within the confines of one or more closely related research fields.

In the 50+ years since *Reviews of Environmental Contamination and Toxicology* (formerly *Residue Reviews)* was first published, the number, scope, and complexity of environmental pollution incidents have grown unabated. During this entire period, the emphasis has been on publishing articles that address the presence and toxicity of environmental contaminants. New research is published each year on a myriad of environmental pollution issues facing people worldwide. This fact, and the routine discovery and reporting of emerging contaminants and new environmental contamination cases, creates an increasingly important function for *Reviews.* The staggering volume of scientific literature demands remedy by which data can be synthesized and made available to readers in an abridged form. *Reviews* addresses this need and provides detailed reviews worldwide to key scientists and science or policy administrators, whether employed by government, universities, nongovernmental organizations, or the private sector.

There is a panoply of environmental issues and concerns on which many scientists have focused their research in past years. The scope of this list is quite broad, encompassing environmental events globally that affect marine and terrestrial ecosystems; biotic and abiotic environments; impacts on plants, humans, and wildlife; and pollutants, both chemical and radioactive; as well as the ravages of environmental disease in virtually all environmental media (soil, water, air). New or enhanced safety and environmental concerns have emerged in the last decade to be added to incidents covered by the media, studied by scientists, and addressed by governmental and private institutions. Among these are events so striking that they are creating a paradigm shift. Two in particular are at the center of ever increasing media as well as scientific attention: bioterrorism and global warming. Unfortunately, these very worrisome issues are now superimposed on the already extensive list of ongoing environmental challenges.

The ultimate role of publishing scientific environmental research is to enhance understanding of the environment in ways that allow the public to be better informed or, in other words, to enable the public to have access to sufficient information. Because the public gets most of its information on science and technology from internet, TV news, and reports, the role for scientists as interpreters and brokers of scientific information to the public will grow rather than diminish. Environmentalism is an important global political force, resulting in the emergence of multinational consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the twenty-first century involve a consortium of technologists and environmentalists, or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls to avoid endangering the environment, public health, and wildlife. Ensuring safety-in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, because the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists. New legislation that will deal in an appropriate manner with this challenge is currently in the making or has been implemented recently, such as the REACH legislation in Europe. These regulations demand scientifically sound and documented dossiers on new chemicals.

Reviews publishes synoptic articles designed to treat the presence, fate, and, if possible, the safety of xenobiotics in any segment of the environment. These reviews can be either general or specific, but properly lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, (eco)toxicology, and regulation. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems may also be appropriate.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are likely in preparation or planned. The field is so very large and the interests in it are so varied that the editor and the editorial board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of anthropogenic chemicals in our surroundings. Thus, manuscripts may encompass case studies from any country. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their scope.

Manuscripts are often contributed by invitation. However, nominations for new topics or topics in areas that are rapidly advancing are welcome. Preliminary communication with the Editor-in-Chief is recommended before volunteered review manuscripts are submitted. *Reviews* is registered in WebofScience™.

Inclusion in the Science Citation Index serves to encourage scientists in academia to contribute to the series. The impact factor in recent years has increased from 2.5 in 2009 to 7.0 in 2017. The Editor-in-Chief and the Editorial Board strive for a further increase of the journal impact factor by actively inviting authors to submit manuscripts.

Amsterdam, The Netherlands Pim de Voogt
August 2018

Contents

Contributors

Hualin Bian College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China
Hunan Provincial Key Laboratory of Rural Ecosystem Health in Dongting Lake Area, Hunan Agricultural University, Changsha, China

Kartik Dhar Global Centre for Environmental Remediation (GCER), Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia
Department of Microbiology, University of Chittagong, Chittagong, Bangladesh

Xiaohui Ding College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China
Hunan Provincial Key Laboratory of Rural Ecosystem Health in Dongting Lake Area, Hunan Agricultural University, Changsha, China

Yu-Bin Ding Joint International Research Laboratory of Reproductive and Development, Department of Reproductive Biology, School of Public Health, Chongqing Medical University, Chongqing, People's Republic of China

Kannan Krishnan Global Centre for Environmental Remediation (GCER), Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia

Youzhi Li College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China

Jia Luo Hunan Academy of Forestry, Changsha, China

Mallavarapu Megharaj Global Centre for Environmental Remediation (GCER), Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia

William Nelson Joint International Research Laboratory of Reproductive and Development, Department of Reproductive Biology, School of Public Health, Chongqing Medical University, Chongqing, People's Republic of China

Bo Ren College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China
Hunan Provincial Key Laboratory of Rural Ecosystem Health in Dongting Lake Area, Hunan Agricultural University, Changsha, China

Gloria Sakwari Department of Environmental and Occupational Health, School of Public Health and Social Sciences, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

Tan Kar Soon Key Laboratory of Marine Biotechnology of Guangdong Province, Shantou University, Shantou, China

Suresh R. Subashchandrabose Global Centre for Environmental Remediation (GCER), Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia

Kadiyala Venkateswarlu Formerly Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, India

Ying-Xiong Wang Joint International Research Laboratory of Reproductive and Development, Department of Reproductive Biology, School of Public Health, Chongqing Medical University, Chongqing, People's Republic of China

Xin Yao College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China
Hunan Provincial Key Laboratory of Rural Ecosystem Health in Dongting Lake Area, Hunan Agricultural University, Changsha, China

Jinrui Yuan College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China
Hunan Provincial Key Laboratory of Rural Ecosystem Health in Dongting Lake Area, Hunan Agricultural University, Changsha, China

Huaiping Zheng Key Laboratory of Marine Biotechnology of Guangdong Province, Shantou University, Shantou, China

Qiaoqiao Zhou College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China

Trends and Health Risks of Dissolved Heavy Metal Pollution in Global River and Lake Water from 1970 to 2017

Youzhi Li, Qiaoqiao Zhou, Bo Ren, Jia Luo, Jinrui Yuan, Xiaohui Ding, Hualin Bian, and Xin Yao

Contents

Youzhi Li and Qiaoqiao Zhou contributed equally to this work.

Y. Li (✉) · Q. Zhou
College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China
e-mail: liyouzhi2004@163.com; 1138189128@qq.com

B. Ren · J. Yuan · X. Ding · H. Bian · X. Yao
College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, China

Hunan Provincial Key Laboratory of Rural Ecosystem Health in Dongting Lake Area, Hunan Agricultural University, Changsha, China
e-mail: renbo@hunau.edu.cn; 592542440@qq.com; 578008729@qq.com; 1434592249@qq.com; 2631088109@qq.com

J. Luo
Hunan Academy of Forestry, Changsha, China
e-mail: 29738648@qq.com

P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology, Volume 251*, Reviews of Environmental Contamination and Toxicology Volume 251,
https://doi.org/10.1007/398_2019_27

Abbreviations

CEC	Council of the European Communities
CR	Cancer risk
CSF	Cancer slope factor
EPA	Environmental Protection Agency
EU	European Union
HI	Hazard quotient index
HQ	Hazard quotient
M-K	Mann-Kendall
MLR	Multiple linear regression
PCA	Principal component analysis
RFD	Reference dose
UNECE	United Nations Economic Commission for Europe
US	United States

1 Introduction

In recent decades, heavy metal pollution has become a global environmental issue and the prime focus of environmental security. Such heavy metals derive from both natural sources, such as rock weathering, and anthropogenic sources, such as mining, manufacturing, fertilizer and pesticide use, and waste discharge (Hu et al. 2015; Huang et al. 2015; Ren et al. 2015; Facchinelli et al. 2001; Muhammad et al. 2011). In the last half of the twentieth century, the global amount of heavy metals released to the environment amounted to 22,000 ton of Cd, 939,000 ton of Cu, 783,000 ton of Pb, and 1,350,000 ton of Zn (Singh et al. 2003). Given their solubility, heavy metals can be dispersed by water and, subsequently, contaminate water ecosystems (Nguyen et al. 2005; Jiang et al. 2012; Ağca et al. 2014). In the Buriganga River (Bangladesh), the dissolved metal concentration amounted to 126 μg L^{-1} of Cd, 805 μg L^{-1} of Pb, 5,274 μg L^{-1} of Cr, and 595 μg L^{-1} of As in 2006 (Bhuiyan et al. 2011). Such high levels of heavy metals in surface water pose a direct threat to human health and require urgent attention, as well as further research (Muhammad et al. 2011; Gao et al. 2016).

In an effort to reduce the health risks associated with heavy metal pollution, action has been taken worldwide to control their sources. Since the 1970s, the Congress of the United States (US) has mandated the Federal Environmental Protection Agency (EPA) to regulate the manufacturing, processing, commercial use, labeling, and disposal of such harmful substances (Babich and Stotzky 1985). During the 1980s, the attention of this organization was directed toward regulating the permitted maximum metal concentrations in fertilizers and the maximum metal loading in agricultural land (Mortvedt 1996). In the 1990s, the European Community (now the European Union [EU]) obligated the collection and treatment of municipal

wastewater and prohibited the disposal of wastewater to surface water (CEC 1991). In the 2000s, the Chinese government prohibited the use of leaded gasoline nationwide and issued stricter local emission standards for coal combustion (Duan and Tan 2013).

Measures that successfully control the sources of heavy metal pollution lead to a reduction in the amount of heavy metals released to the environment and, eventually, reduce the metal concentrations in surface water. However, to date, no published research has reported on global levels of heavy metal pollution in water bodies such as rivers and lakes. Therefore, this study collected concentrations of 12 dissolved heavy metals (Cd, Pb, Cr, Zn, Cu, Ni, Mn, Fe, Hg, Al, As, and Co) in global river and lake water bodies from published papers and investigated the trends and health risks from 1970 to 2017 (note, data for the latter four metals were insufficient for analysis). The aim of the study was to explore the sources of heavy metal pollution on decadal and continental scales, to assess the effects of implemented countermeasures for pollution control, and to determine successful measures that could be adopted worldwide.

2 Materials and Methods

2.1 Data Collection

Data on the dissolved concentrations of 12 heavy metal species (Cd, Pb, Cr, Zn, Cu, Ni, Mn, Fe, Hg, Al, As, and Co) in river and lake water worldwide were collected initially from published papers (using a search conducted in Google Scholar and Web of Science). Each sample was assigned a specific year according to the reported sampling date, as follows: for a single sampling year, that year was used; for a sampling range of 1–2 years, the first year was used; and for a sampling range of more than 2 years, the middle year was used. When the sampling date was not provided, the year prior to publication was used. The samples we reviewed had been collected from a total of 120 rivers and 116 lakes in Africa, Asia, Europe, North America, Oceania, and South America and selected from pristine areas, polluted areas, urban area, and estuarine area over the period 1970–2017 (Tables 1, 2, and 3).

2.2 Trend Assessment

In our study, we employed the Mann-Kendall (M-K) test (Mann 1945; Kendall 1975), used extensively to detect change trends in heavy metal pollution over time (Gao et al. 2016; Sharley et al. 2016). As the amount of collected data (number of rivers or lakes) changed by year, the data were classified into five decadal groups (1970–1979, 1980–1989, 1990–1999, 2000–2009, and 2010–2017) to improve the exploration of the trends in dissolved heavy metal pollution in water. The mean

Table 1 Regional distribution of the rivers and lakes considered in this study

Continents	Rivers		Lakes	
	Number	Name of typical rivers	Number	Name of typical lakes
Africa	13[a]	Congo River, Niger River, Nile River, Nyando River, Nzoia River	4[b]	Kainji Lake, Nasser Lake, Victoria Lake
Asia	75[c]	Aras River, Brahmaputra River, Buriganga River, Ganga River, Lean River, Mekong River, Ob River, Pearl River, Pechora River, Tigris River, Yangtze River, Yellow River, Yenisey River	97[d]	Ataturk Dam Lake, Bolgoda Lake, Chaohu Lake, Dongting Lake, Hazar Lake, Hussainsagar Lake, Poyang Lake, Qinghaihu Lake, Taihu Lake, Tasik Chini Lake
Europe	18[e]	Arno River, Danube River, Dordogne River, Elbe River, Mersey River, Rhône River, Stour River, Tiber River	11[f]	Balaton Lake, Hampen Lake, Sortesø Lake, Venice Lagoon
North America	4[g]	Arkansas River, Mississippi River, Tippecanoe River	4[h]	Ivanhoe Lake, Palestine Lake, Thompson Lake
South America	7[i]	Amazon River, Orinoco River, Paraiba do Sul-Guandu River, Pilcomayo River, Sinos River	–	–
Oceania	3[j]	South Alligator River, South Esk River, St. Paul's River	–	–

[a]Dorten et al. (1991), Lalah et al. (2008), Dupré et al. (1996), Banzi et al. (2015), Krika and Krika (2017), and Faton et al. (2015)

[b]Rashed (2001), Muwanga and Barifaijo (2006), and Oyewale and Musa (2006)

[c]Polprasert (1982), Cenci and Martin (2004), Elbaz-Poulichet et al. (1987), Huang et al. (1988), Guay et al. (2010), Martin et al. (1993), Guieu et al. (1996), Dai and Martin (1995), Bradley and Woods (1997), Shiller and Boyle (1987), Cui et al. (2011), Li et al. (2013), Wan et al. (2007), Wang et al. (2018), Varol and Şen (2012), Luo (1984), Sin et al. (1991), Zingde et al. (1988), Shen et al. (1989), Karadede-Akin and Ünlü (2007), Demirak et al. (2006), Karbassi et al. (2008), Kar et al. (2008), Aydinalp et al. (2005), Fan et al. (2008), Turgut (2003), Reza and Singh (2010), Sundaray (2010), Konhauser et al. (1997), Salati and Moore (2010), Varol (2013), Varol et al. (2010), Rahman et al. (2014), Kumar et al. (2013), Li and Zhang (2010), Wu et al. (2002), Zeng et al. (2002), Zhang and Hu (2006), Li and Liu (2009), Cheng and Li (2017), Wang et al. (2015), Li et al. (2008, 2010), Yang et al. (2008), Su et al. (2006), Cheng et al. (2009), Sun et al. (2009), Deng et al. (2016), Qin et al. (2015), Zhang et al. (2016), Gümgüm et al. (1994), Khan et al. (2005), Bhuiyan et al. (2011), Sharma and Vaishnav (2015), Ismail et al. (2013), Rahman et al. (2015), Arefin et al. (2016), Zilkir et al. (2006), Chen and Zhang (1986), Shi (2014), Li (2009), Gong (2011), and Nasehi et al. (2012)

[d]Özmen et al. (2004), Ebrahimpour and Mushrifah (2008), Pathiratne et al. (2009), Barlas et al. (2005), Singare et al. (2010), Jiang et al. (2012), Tao et al. (2012), Rahman et al. (2014), Liu et al. (2010, 2011), Yue et al. (2015), Li et al. (2010, 2013), Wang et al. (2014a, b), Lu et al. (2016), Tian et al. (2011), Yan et al. (2018), Sun and Zang (2012), Mao et al. (2013), Wang et al. (2018), Yang et al. (2008), Sun et al. (2009), Wu et al. (2018), Karadede and Ünlü (2000), Reddy et al. (2012), Farkas et al. (2000), Alhas et al. (2009), Singare et al. (2013), Moore et al. (2009), Zhang (2013), and Meng (2016)

[e]Müller and Förstner (1975), Zwolsman and van Eck (1999), Guieu et al. (1998), Martin et al. (1993, 1994), Dorten et al. (1991), Stoica (1999), Elbaz-Poulichet et al. (1987, 1996), Pettine et al.

(continued)

(1996), Bonanno and Giudice (2010), Bubb and Lester (1994), Say et al. (1981), Adamiec and Helios-Rybicka (2002), and Ramos et al. (1999)
[f]Schierup and Larsen (1981), Martin et al. (1994), Nguyen et al. (2005), and Waara (1992)
[g]Adams et al. (1980), Presley et al. (1980), Martin et al. (1993), Shiller and Boyle (1987), Winner et al. (1980), DeLeon et al. (1986), and Kimball et al. (1995)
[h]Adams et al. (1980), Shephard et al. (1980), Yousef et al. (1984), and McFarlane and Franzin (1978)
[i]Martin et al. (1993), Malm et al. (1988), Shiller and Boyle (1987), Smolders et al. (2003), Hatje et al. (1998), Miller et al. (2004), and Magdaleno et al. (2014)
[j]Thorp and Lake (1973) and Munksgaard and Parry (2001)

dissolved metal concentration in each decadal group was determined as the average of all the collected data in that decadal group. As we inly identified only 92 data points for As, 51 for Co, 38 for Hg, and 7 for Al, these four metals were subsequently removed from the database. M-K tests were conducted on the remaining eight metals (Cd, Pb, Cr, Zn, Cu, Ni, Mn, and Fe) to ensure accuracy of the results (Table 2). We used the M-K calculation methods described by Kisi and Ay (2014) and we used 95% two-tailed confidence levels.

The mean dissolved metal concentrations for each continent were determined as the average of all the collected data for that continent. As the data from Oceania reflected only three rivers (South Alligator River, South Esk River, and St. Paul's River) and there were only three data points for Zn, Cu, and Fe, two data points for Cd and Mn, one data for Pb and Ni, and no data for Cr, this continent was excluded; therefore, only data from Africa, Asia, Europe, North America, and South America were selected to compare the mean dissolved metal concentrations.

2.3 Health Risk Assessment

Humans are exposed to heavy metals via three main pathways: oral ingestion, mouth and nose inhalation, and dermal absorption; ingestion and dermal absorption are the most common pathways for the heavy metal pollution in water (Li and Zhang 2010; Muhammad et al. 2011). The health risk associated with heavy metal toxicity is characterized into non-carcinogenic and carcinogenic. Non-carcinogenic risk, reflected by the hazard quotient index (HI), is defined as the sum of the hazard quotient (HQ) from both exposure routes (oral ingestion and dermal contact). For each exposure route, the HQ is estimated by the average intake of heavy metals from that route divided by the corresponding reference dose (RFD; i.e., the security threshold of a specific metal). When the HI exceeds one, there could be an adverse non-carcinogenic effect on human health. Similarly, carcinogenic risk, reflected by the cancer risk (CR), is the probability of an individual developing any type of cancer over a lifetime and is defined as the sum of CR from both exposure routes. For each exposure route, the CR is assessed as the average intake of heavy metals in that exposure multiplied by the corresponding cancer slope factor (CSF).

Table 2 Dissolved metal concentrations (μg L^{-1}), total sample number (TSN), pristine sample number (PRSN), and polluted sample number (POSN) in global combined river and lake water and Mann-Kendall (MK) test results from the 1970s to 2010s

	1970s				1980s				1990s			
Metals	Mean ± SD	TSN	PRSN	POSN	Mean ± SD	TSN	PRSN	POSN	Mean ± SD	TSN	PRSN	POSN
Cd	9.22 ± 20.61	18	5	15	0.85 ± 2.38	42	14	37	1.05 ± 2.24	29	11	19
Pb	19.13 ± 45.12	7	2	6	11.38 ± 24.48	33	11	28	36.11 ± 84.67	28	11	18
Cr	45.87 ± 71.86	6	0	6	2.28 ± 4.28	11	3	10	9.14 ± 11.51	9	2	7
Zn	233.79 ± 471.83	19	4	17	74.24 ± 230.58	34	11	29	70.81 ± 102.75	23	7	16
Cu	36.51 ± 87.04	17	5	14	4.76 ± 6.11	37	12	32	13.11 ± 32.14	31	11	21
Ni	2.33 ± 3.87	6	2	4	2.76 ± 6.91	17	8	12	45.24 ± 173.48	18	5	14
Mn	165.01 ± 254.78	7	1	7	694.04 ± 607.71	3	0	3	170.89 ± 162.27	9	0	9
Fe	26.97 ± 24.75	8	2	7	222.35 ± 552.27	13	6	10	39.69 ± 81.55	9	2	7
	2000s				2010s				1970–2017			
Metals	Mean ± SD	TSN	PRSN	POSN	Mean ± SD	TSN	PRSN	POSN	MK test	TSN	PRSN	POSN
Cd	10.04 ± 33.77	89	12	88	16.18 ± 52.32	42	11	35	0.24	220	53	194
Pb	26.24 ± 87.07	94	12	92	58.66 ± 113.53	40	12	32	−0.24	202	48	176
Cr	126.14 ± 634.82	85	9	84	130.56 ± 445.50	43	22	25	0.24	154	36	132
Zn	118.42 ± 376.74	111	10	108	482.67 ± 1,384.96	41	12	33	−0.73	228	44	203
Cu	34.14 ± 91.82	114	13	111	62.45 ± 212.25	52	11	43	0.24	251	52	221
Ni	40.32 ± 93.77	74	6	72	201.20 ± 656.54	34	11	24	1.22	149	32	126
Mn	180.17 ± 478.27	70	9	68	137.62 ± 171.88	24	14	12	0.24	113	24	99
Fe	553.70 ± 1,412.86	68	7	66	924.81 ± 1,693.36	28	15	15	0.73	126	32	105

Table 3 Dissolved metal concentrations (μg L^{-1}), total sample number (TSN), pristine sample number (PRSN), and polluted sample number (POSN) in global combined river and lake water of five continents from 1970 to 2017

	Africa				Asia				Europe			
Metals	Mean ± SD	TSN	PRSN	POSN	Mean ± SD	TSN	PRSN	POSN	Mean ± SD	TSN	PRSN	POSN
Cd	3.00 ± 2.93	12	3	11	10.71 ± 38.31	150	43	128	0.62 ± 1.28	34	3	33
Pb	34.05 ± 35.23	16	3	15	36.05 ± 98.45	147	41	123	7.57 ± 15.68	28	3	27
Cr	36.94 ± 68.78	11	4	10	128.04 ± 583.13	125	31	104	7.25 ± 9.63	6	1	6
Zn	59.18 ± 65.21	16	2	16	208.05 ± 784.50	158	35	136	96.07 ± 332.16	28	3	27
Cu	34.17 ± 58.42	15	1	14	37.59 ± 134.33	183	44	157	8.48 ± 15.90	29	3	28
Ni	29.73 ± 39.21	13	1	12	91.62 ± 380.75	111	28	91	3.95 ± 4.34	12	1	12
Mn	517.55 ± 898.01	12	0	12	126.26 ± 269.06	86	22	72	75.82 ± 77.23	5	1	5
Fe	1,240.99 ± 1,897.86	5	1	4	566.34 ± 1,424.80	98	28	79	52.17 ± 110.60	5	0	5
	North America				South America							
Metals	Mean ± SD	TSN	PRSN	POSN	Mean ± SD	TSN	PRSN	POSN				
Cd	3.64 ± 4.92	14	2	13	2.71 ± 5.96	8	2	7				
Pb	24.83 ± 47.07	6	0	6	32.50 ± 48.35	5	1	5				
Cr	26.64 ± 64.29	8	0	8	12.48 ± 15.35	4	1	4				
Zn	196.93 ± 327.95	15	2	14	83.47 ± 130.65	8	2	7				
Cu	38.13 ± 99.97	13	2	12	15.35 ± 31.19	8	2	7				
Ni	5.84 ± 10.37	7	1	6	10.87 ± 23.37	5	1	4				
Mn	377.54 ± 518.82	6	1	6	69.00 ± 76.37	2	0	2				
Fe	31.12 ± 51.32	11	2	10	1,158.21 ± 1,213.62	4	1	4				

The calculation methods for the average intake of metals from oral ingestion or dermal absorption and the relevant parameters (RFD, CSF) used here were described by Li and Zhang (2010) and Gao et al. (2016). The non-carcinogenic risk of eight metals (Cd, Pb, Cr, Zn, Cu, Ni, Mn, and Fe) was estimated relevant to the five decades and five continents by using their corresponding mean concentrations in water (Liu et al. 2015; Gao et al. 2016). Owing to a lack of relevant references for some carcinogenic metals (Cd, Pb, Ni, and Cr), only Pb and Cr were selected to estimate their carcinogenic risk relevant to oral ingestion; their carcinogenic risk relevant to dermal absorption was not assessed (De Miguel et al. 2007; Li and Zhang 2010; Liu et al. 2015).

2.4 Source Apportionment

Principal component analysis (PCA) followed by multiple linear regression (MLR) is a useful method for source apportionment (Yang et al. 2017; Ashayeri et al. 2018; Larsen and Baker 2003). In this study, PCA-MLR was used to determine the contribution percentages of the investigated metal sources to water pollution. First, PCA was employed to represent the total variability of the original metal data in a minimum number of factors; that is, factors with an eigenvalue greater than one were extracted (Loska and Wiechuła 2003). The metal source responsible for each factor could be identified by critically evaluating the factor loadings (Wuana and Okieimen 2011; Järup 2003). Subsequently, MLR was conducted using the standardized PCA scores and the standardized normal deviation of the total dissolved metal concentrations as the independent and dependent variables, respectively. Regression coefficients were applied subsequently to estimate the contribution percentages of the various metal sources.

To compare the changes in the metal pollution sources over time, the potential sources in water were classified into four main types, namely, rock weathering, fertilizer and pesticide use, mining and manufacturing, and waste discharge. Source apportionment of metal pollution in river and lake water was conducted using the SPSS V17.0 software (IBM Corp., Armonk, NY, USA).

3 Results

3.1 Trends of Dissolved Heavy Metal Pollution in Water

The concentrations of the dissolved heavy metals in water differed between the five different time groups over the period 1970–2017 (Table 2 and Fig. 1). Most heavy metal species had the highest dissolved concentrations in the 2010s and the lowest concentrations in the 1970s or 1980s. Collectively, increasing trends were shown in the water for Cd, Cr, Cu, Ni, Mn, and Fe and decreasing trends for Pb and Zn.

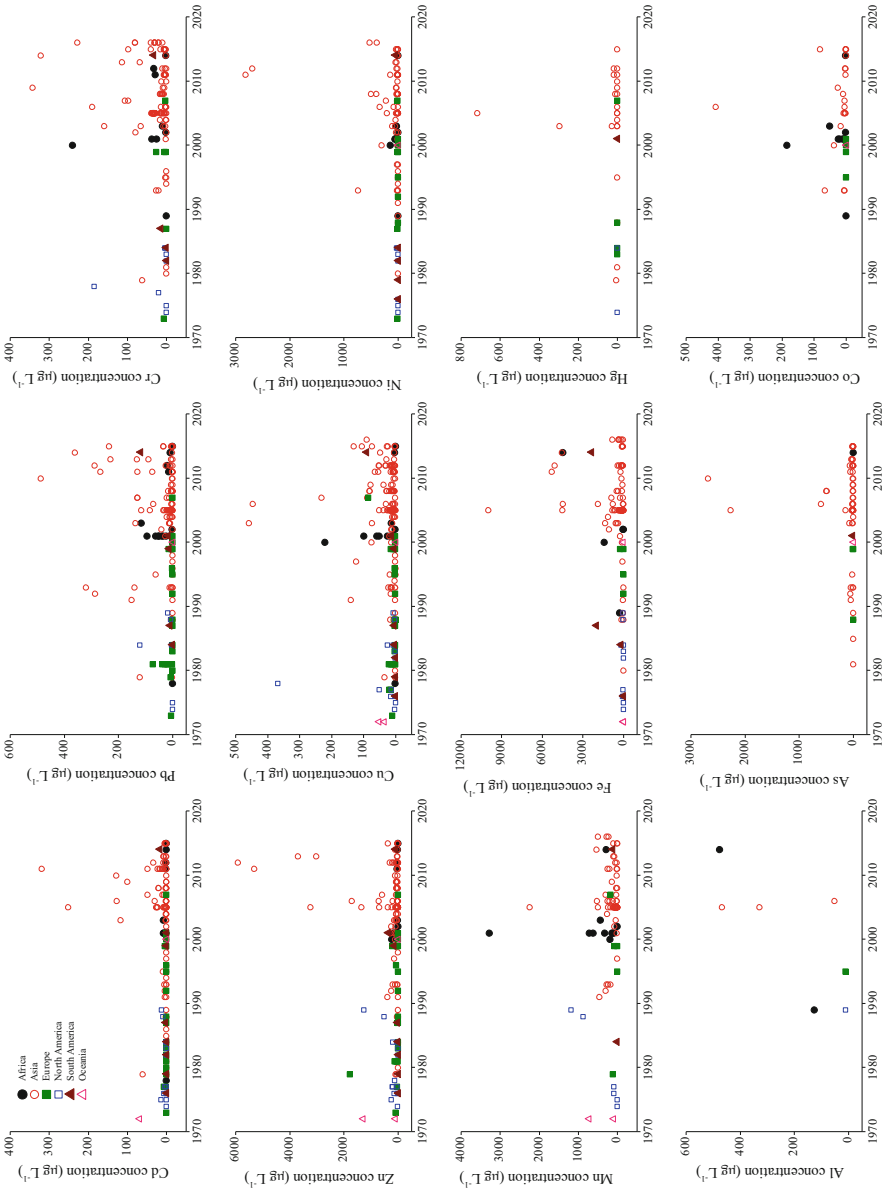

Fig. 1 Dissolved metal concentrations in water by continent from 1970 to 2017 (points represent the mean in one river or lake for 1 year)

The concentrations of dissolved heavy metals in water also differed for the five continents (Table 3 and Fig. 1). Most heavy metal species showed the highest dissolved concentrations in Asia and the lowest in Europe.

3.2 Human Health Risks of Dissolved Heavy Metals in Water

The hazard quotient indices of most heavy metals relevant to non-carcinogenic risks were generally lower than one (Table 4). However, these indices were greater than one for Pb in the 2010s, for Cr in the 2000s and 2010s, and for Zn in the 1970s, 2000s, and 2010s, as well as for Cr in Asia, and for Zn in Asia and North America.

The cancer risks related to Pb contamination were all in the secure range (10^{-6}–10^{-4}) on the five continents over the five decades. However, the cancer risks associated with Cr contamination exceeded the hazardous level in the 1970s, 2000s, and 2010s, as well as in Africa, Asia, and North America for the entire period.

3.3 Sources of Dissolved Heavy Metal Pollution in Water

The main pollution sources in water have changed significantly over time (Table 5). In the 1970s, the main metal sources were fertilizer and pesticide use, along with mining and manufacturing, with a total contribution exceeding 64%. In the 1980s, with the same sources as in the previous decade, the total contribution increased to more than 78%. In the 1990s, the main sources were mining and manufacturing, along with rock weathering, with a total contribution exceeding 58%. In the 2000s, with the main sources remaining the same, the total contribution exceeded 46%. In the 2010s, with the dominant sources the same as those in the 2000s, the combined contribution increased to more than 65%.

Furthermore, the main pollution sources in water differed by continent (Table 6). In Africa, the main metal sources were waste discharge and rock weathering, with a total contribution exceeding 59%. In Asia and South America, the main sources were mining and manufacturing, along with rock weathering, with total contributions exceeding 53% and 60%, respectively. In Europe, mining and manufacturing, waste discharge, and rock weathering were all dominant sources, with a total contribution of 97%. In North America, fertilizer and pesticide use, along with rock weathering, were the main sources, with a total contribution exceeding 86%.

4 Discussion

From 1970 to 2017, river and lake water worldwide showed an increasing trend in the concentrations of dissolved Cd, Cr, Cu, Ni, Mn, and Fe and a decreasing trend for Pb and Zn. This indicated that the heavy metal loadings in water had increased for

Table 4 Non-carcinogenic risk (hazard quotient index) of each metal and carcinogenic risk (cancer risk) of Pb and Cr over five decades and five selected continents

	Decades					Continents				
Metals	1970s	1980s	1990s	2000s	2010s	Africa	Asia	Europe	North America	South America
Cd	5.58E-01	5.14E-02	6.35E-02	6.08E-01	9.79E-01	1.82E-01	6.48E-01	3.75E-02	2.20E-01	1.64E-01
Pb-non cancer	3.75E-01	2.23E-01	7.08E-01	5.14E-01	1.15E+00	6.67E-01	7.07E-01	1.48E-01	4.87E-01	6.37E-01
Cr-non cancer	5.06E-01	2.52E-02	1.01E-01	1.39E+00	1.44E+00	4.08E-01	1.41E+00	8.00E-02	2.94E-01	1.38E-01
Zn	2.14E+00	6.78E-01	6.47E-01	1.08E+00	4.41E+00	5.41E-01	1.90E+00	8.77E-01	1.80E+00	7.62E-01
Cu	2.51E-02	3.27E-03	9.02E-03	2.35E-02	4.30E-02	2.35E-02	2.59E-02	5.83E-03	2.62E-02	1.06E-02
Ni	3.28E-03	3.88E-03	6.36E-02	5.67E-02	2.83E-01	4.18E-02	1.29E-01	5.55E-03	8.21E-03	1.53E-02
Mn	1.91E-01	8.03E-01	1.98E-01	2.08E-01	1.59E-01	5.99E-01	1.46E-01	8.77E-02	4.37E-01	7.98E-02
Fe	1.06E-03	8.73E-03	1.56E-03	2.17E-02	3.63E-02	4.87E-02	2.22E-02	2.05E-03	1.22E-03	4.54E-02
Pb-caner	1.91E-06	1.14E-06	3.60E-06	2.62E-06	5.85E-06	3.40E-06	3.60E-06	7.56E-07	2.48E-06	3.24E-06
Cr-cancer	2.69E-04	1.34E-05	5.37E-05	7.41E-04	7.66E-04	2.17E-04	7.52E-04	4.26E-05	1.56E-04	7.33E-05

Table 5 Rotated component matrix of selected metal concentrations in global combined river and lake water by decade

	1970s			1980s			1990s			2000s			2010s		
Metals	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Cd	0.93	−0.03	0.08	0.94	0.15	−0.04	0.82	−0.08	0.02	0.30	0.86	−0.01	0.68	−0.02	0.01
Pb	0.04	−0.24	0.67	0.07	0.89	−0.09	0.78	0.13	0.34	0.88	0.15	0.10	0.21	0.07	0.31
Cr	−0.10	0.89	0.31	−0.13	−0.09	0.71	0.07	0.80	−0.26	0.83	0.13	0.15	0.94	0.05	0.11
Zn	0.56	0.05	−0.04	0.93	0.00	−0.11	−0.01	0.15	0.91	−0.02	0.90	0.18	0.69	−0.03	0.67
Cu	0.10	0.87	−0.08	0.06	0.93	0.05	0.43	−0.11	0.42	0.13	0.12	0.77	−0.14	−0.09	0.96
Ni	0.09	0.28	0.65	0.17	−0.40	0.36	0.83	0.27	−0.32	0.61	0.48	0.10	0.95	0.01	0.09
Mn	0.84	−0.01	0.02	0.92	−0.09	−0.03	0.03	−0.84	−0.10	0.41	−0.01	0.24	−0.02	0.93	−0.08
Fe	−0.08	0.16	0.73	−0.06	0.03	0.86	0.10	0.81	0.26	0.21	0.06	0.81	0.03	0.93	0.08
Eigenvalue	1.98	1.92	1.24	2.73	1.89	1.29	2.52	1.85	1.33	3.10	1.24	1.01	3.03	1.75	1.25
Cumulative percentage	24.72	48.69	64.18	34.18	57.81	73.97	31.50	54.58	71.22	38.80	54.25	66.81	37.84	59.75	75.36
Possible source[a]	FP/ MM	MM/ RW	WD/ RW	FP/ MM	FP/ WD	MM/ RW	WD/ FP	MM/ RW	FP/ RW	MM/ WD	FP	MM/ RW	MM/ WD	MM/ RW	FP/ RW
Contribution percentage	64.17	18.10	17.73	78.14	3.33	18.54	18.97	58.27	22.76	34.60	19.86	45.54	19.44	64.58	15.98

[a]*RW* rock weathering, *FP* fertilizer and pesticide use, *MM* mining and manufacturing, *WD* waste discharge

Table 6 Rotated component matrix of selected metal concentrations in combined river and lake water by continent

	Africa				Asia			Europe			North America			South America	
Metals	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2
Cd	−0.16	0.80	−0.11	0.08	0.70	0.28	−0.02	0.20	0.75	0.22	0.96	0.03	0.11	0.96	−0.02
Pb	−0.04	0.28	0.53	−0.20	0.08	0.89	0.05	0.58	0.33	0.01	0.08	0.13	−0.68	0.89	−0.03
Cr	0.04	0.11	−0.04	0.96	0.16	0.83	0.07	0.66	−0.16	0.42	0.00	0.97	0.09	0.92	0.02
Zn	0.32	0.77	0.10	0.03	0.87	0.12	0.03	0.15	−0.08	−0.64	0.94	−0.01	−0.12	−0.02	0.42
Cu	0.91	0.27	−0.01	−0.18	0.12	0.46	0.03	−0.03	0.82	−0.26	0.00	0.98	0.08	0.94	−0.09
Ni	0.89	−0.18	−0.07	0.26	0.84	0.07	0.06	0.94	0.05	−0.05	0.18	0.10	0.55	0.04	0.92
Mn	−0.05	0.00	0.85	−0.06	−0.02	−0.01	0.93	0.10	−0.11	0.79	0.92	−0.02	0.03	−0.72	0.48
Fe	0.02	−0.29	0.67	0.19	0.08	0.14	0.91	0.76	0.08	−0.17	−0.09	0.26	0.63	0.79	0.41
Eigenvalue	1.90	1.47	1.41	1.07	2.62	1.65	1.24	2.40	1.52	1.13	2.69	2.09	1.10	4.61	1.40
Cumulative percentage	23.77	42.15	59.84	73.18	32.80	53.45	68.98	30.01	49.03	63.13	33.63	59.74	73.55	57.67	75.12
Possible source[a]	MM/RW	FP	WD/RW	MM	FP/WD	MM/WD	MM/RW	MM/WD	FP	MM/RW	FP/RW	MM	WD/RW	FP/WD	MM/RW
Contribution percentage	2.93	21.32	59.15	16.60	22.06	25.18	52.77	48.07	3.13	48.81	85.74	7.40	6.86	40.50	59.50

[a]*RW* rock weathering, *FP* fertilizer and pesticide use, *MM* mining and manufacturing, *WD* waste discharge

the former and decreased for the latter over that period. This finding showed the poor results obtained and illustrated the failure of the measures to control metal pollution. Furthermore, the dissolved heavy metal concentrations differed by continent. As a typical developed region, Europe had the lowest dissolved concentration for most heavy metal species (Cd, Pb, Cr, Cu, Ni, and Mn). In contrast, developing regions such as Asia had the highest dissolved concentration for Cd, Pb, Cr, Zn, and Ni. This indicates not only that the measures to control pollution have been more successful in Europe than in Asia but also that such measures should be extended to other regions.

The health risk assessment showed that the hazard quotient indices of most heavy metal species (Cd, Cu, Ni, Mn, and Fe) were smaller than one, suggesting that these metals posed negligent non-carcinogenic risks to human health. However, the hazard quotient indices of other heavy metal species, such as Pb (in the 2010s), Cr (in the 2000s and 2010s), and Zn (in the 1970s, 2000s, and 2010s), were greater than one, implying that these metals could have caused adverse non-carcinogenic effects to human health in those decades. As regards the two selected carcinogenic heavy metals, the cancer risks associated with Pb from oral ingestion were lower than the hazardous level for the five decades and five continents. However, the cancer risks related to Cr exceeded the hazardous level in the 1970s, 2000s, and 2010s, as well as in Africa, Asia, and North America over the entire period. As exposure to Cr is associated with a high risk of contracting cancer and as there are other pathways (dermal contact and inhalation) to heavy metal exposure in addition to the oral ingestion considered in the present study, preventing such exposure should be a matter of high concern (De Miguel et al. 2007; Li and Zhang 2010; Liu et al. 2015). In addition to water, exposure to heavy metals from the food chain (vegetables, rice, fruits, fish, and other food) could lead to the accumulation of such metals, ultimately leading to chronic toxic effects in humans (Yi et al. 2011; Gao et al. 2016). Therefore, the actual health risks of heavy metal exposure to humans could be more substantial than the results this assessment indicates. Consequently, this should be a matter of global concern.

Source apportionment showed that the main heavy metal sources in river and lake water have changed over time. From the 1970s to the 1980s, these sources were mining and manufacturing, along with fertilizer and pesticide use. A study in 1981–1983 found that mineral refining was the main source for Cd, Hg, Pb, Cu, and Zn pollution in surface water in the National Park of Doñana (Baluja et al. 1985). From the 1990s to the 2010s, the sources were mining and manufacturing, along with rock weathering. A study in 2003 showed that sources of Cd and Pb in Kumho River (Korea) originated from mine discharge (Kim et al. 2010). This finding suggests mining and manufacturing as the critical sources to control global heavy metal pollution in river and lake water. Additionally, the metal sources differed significantly by continent, with waste discharge and rock weathering being dominant in Africa; mining and manufacturing, along with rock weathering being dominant in Asia and South America; fertilizer and pesticide use, along with rock weathering being dominant in North America; and mining and manufacturing, waste discharge, and rock weathering being dominant in Europe. For instance, in the Obuasi mining

area of Ghana, Pb in surface water derives from waste discharge from towns and mine machinery maintenance yards (Armah et al. 2010). In the Yangtze River, heavy metals (Cu, Ni, Fe, Co, and Al) are mainly derived from rock weathering (Wang et al. 2011). Therefore, regional pollution-control measures should concentrate on such region-specific sources.

The decreasing trend in the concentrations of dissolved Pb and Zn in river and lake water indicate that the pollution-control measures for these substances had produced positive effects. For example, from 1994, the US EPA Part 503 rule restricted the ceiling concentration of Pb (300 mg kg^{-1}) and Zn (2,800 mg kg^{-1}) in biosolids applied to the land (Agency 1994). In 1998, the United Nations Economic Commission for Europe (UNECE) agreed the Aarhus Protocol on Heavy Metals to control harmful levels of Pb in Europe (Duan and Tan 2013). In China, after the nationwide prohibition of leaded gasoline in 2000, the Pb pollution level decreased remarkably in cities (Wang et al. 2003). Additionally, having the lowest dissolved metal concentration and the lowest health risk demonstrates the efficiency of the pollution-control measures in Europe; we suggest that these measures should be adopted by the rest of the world. Since the last century, the maximum heavy metal concentration permitted in fertilizers has been regulated in Europe. For instance, in the mid-1990s, the Dutch government proposed regulations limiting the maximum Cd concentration in P fertilizers to 35 mg kg^{-1} (Anon 1989). In addition to the maximum Cd concentration of 100 mg kg^{-1} in P fertilizers, Sweden imposed a tax (30 SEK kg^{-1} Cd) on P fertilizers with Cd concentrations between 5 and 100 mg kg^{-1}. The EU placed limits on the metal concentrations in industrial effluents discharged into the Rhine River in an effort to alleviate the metal pollution in the river (Mortvedt 1996). In 1998, the United Nations Economic Commission for Europe signed the Aarhus Protocol on Heavy Metals to control harmful levels of Cd (Duan and Tan 2013). European legislation prescribes the priority order to be applied to waste treatment, such as prevention, reuse, recycling, other recovery (e.g., energy recovery), and disposal (Kelessidis and Stasinakis 2012). These measures, including implementing rigorous standards on metal emissions, limiting the metals added to products, and pretreating metal-contaminated waste effectively, have controlled heavy metal pollution in rivers and lakes and, we suggest, should be extended worldwide.

5 Conclusions

The present study clearly shows that global river and lake water have increasing trends for Cd, Cr, Cu, Ni, Mn, and Fe and decreasing trends for Pb and Zn over the past period from 1970 to 2017. Most heavy metals had low non-carcinogenic risks over this period. The cancer risks associated with Pb were lower than the hazardous level on the five continents over the five decades, whereas the cancer risks related to Cr exceeded the hazardous level in the 1970s, 2000s, and 2010s, as well as in Africa, Asia, and North America over the entire period. This finding illustrates the failure of

measures to control global metal pollution; the high cancer risks related to Cr on these continents should be a matter of high concern.

Over the past five decades, mining and manufacturing were consistently found to be critical sources of metal pollution in river and lake water. The heavy metal sources differed significantly by continent, with waste discharge and rock weathering dominant in Africa; mining and manufacturing, along with rock weathering dominant in Asia and South America; fertilizer and pesticide use, along with rock weathering dominant in North America; and mining and manufacturing, waste discharge, and rock weathering dominant in Europe. Therefore, regional pollution-control measures should concentrate on such region-specific sources.

The lowest mean dissolved concentrations of most heavy metals in Europe suggest that the countermeasures in the continent have successfully controlled heavy metal pollution. Successful measures include implementing rigorous standards for metal emissions, limiting the metal concentrations in products, and rigorously treating metal-contaminated waste. In addition to such environmental measures, ecological approaches should be considered for heavy metal treatment. For example, after landscape reclamation in catchments, heavy metal inputs to the United Kingdom's Fendrod Lake declined (Blake et al. 2007). Therefore, comprehensive application of environmental and ecological measures should be considered for the remediation of heavy metal-polluted rivers and lakes.

6 Summary

Heavy metal pollution in surface water is a global environmental problem. This study analyzed the trends, health risks, and sources of eight dissolved heavy metal species in river and lake water across five continents (Africa, Asia, Europe, North America, and South America; Oceania was excluded owing to a lack of data) for the period 1970–2017. We wanted to assess the effects of various implemented countermeasures to pollution and to determine those that could be adopted worldwide. Collectively, the water system showed increasing trends for Cd, Cr, Cu, Ni, Mn, and Fe and decreasing trends for Pb and Zn. The mean dissolved concentrations of most heavy metals were highest in Asia and lowest in Europe. Most heavy metals had low non-carcinogenic risks over this period. The cancer risks associated with Pb were lower than the hazardous level on all five continents over the five decades, whereas the cancer risks related to Cr exceeded the hazardous level in the 1970s, 2000s, and 2010s, and in Africa, Asia, and North America over the entire period. Mining and manufacturing were consistently found to be critical sources of metal pollution from 1970 to 2017. However, the heavy metal sources differed significantly by continent, with waste discharge and rock weathering dominant in Africa; mining and manufacturing, along with rock weathering, are dominant in Asia and South America; fertilizer and pesticide use, along with rock weathering, are dominant in North America; and mining and manufacturing, waste discharge, and rock weathering are dominant in Europe. Global trends in the metal loadings in water and in relevant

pollution-control measures suggest that countermeasures in Europe have successfully controlled heavy metal pollution. The successful measures include implementing rigorous standards for metal emissions, limiting the metal concentrations in products, and rigorously treating metal-contaminated waste. Therefore, the measures implemented in Europe should be extended worldwide to treat heavy metal pollution in water.

Acknowledgments The authors greatly appreciate Marco Vighi, associate editor of Reviews of Environmental Contamination and Toxicology, and two anonymous reviewers for the constructive suggestions on this manuscript. The study was supported financially by the Key Research and Development Program of Hunan Province (2017WK2082; 2017SK2300; 2017NK2383), the Scientific Research Fund of Hunan Provincial Education Department (16B121), and the Science and Technology Program of the Forestry Department of Hunan Province (XKL201702).

Conflict of Interest The authors declare that they have no conflict of interest.

References

Adamiec E, Helios-Rybicka E (2002) Distribution of pollutants in the Odra river system. Part IV. Heavy metal distribution in water of the upper and middle Odra River, 1998-2000. Pol J Environ Stud 11(6):669–674

Adams TG, Atchison GJ, Vetter RJ (1980) The impact of an industrially contaminated lake on heavy metal levels in its effluent stream. Hydrobiologia 69(1–2):187–193

Ağca N, Karanlık S, Ödemiş B (2014) Assessment of ammonium, nitrate, phosphate, and heavy metal pollution in groundwater from Amik Plain, Southern Turkey. Environ Monit Assess 186(9):5921–5934

Agency USEP (1994) A plain English guide to the EPA part 503 biosolids rule. Washington

Alhas E, Oymak SA, Akin HK (2009) Heavy metal concentrations in two barb, *Barbus xanthopterus* and *Barbus rajanorum mystaceus* from Atatürk Dam Lake, Turkey. Environ Monit Assess 148(1–4):11–18

Anon (1989) Cadmium in phosphates: one part of a wider environmental problem. Phosphorus Potassium 162(4):23–30

Arefin MT, Rahman MM, Wahid-U-Zzaman M, Kim JE (2016) Heavy metal contamination in surface water used for irrigation: functional assessment of the Turag River in Bangladesh. J Appl Biol Chem 59(1):83–90

Armah FA, Obiri S, Yawson DO, Onumah EE, Yengoh GT, Afrifa EKA, Odoi JO (2010) Anthropogenic sources and environmentally relevant concentrations of heavy metals in surface water of a mining district in Ghana: a multivariate statistical approach. J Environ Sci Health A 45(13):1804–1813

Ashayeri NY, Keshavarzi B, Moore F, Kersten M, Yazdi M, Lahijanzadeh AR (2018) Presence of polycyclic aromatic hydrocarbons in sediments and surface water from Shadegan wetland-Iran: a focus on source apportionment, human and ecological risk assessment and sediment-water exchange. Ecotoxicol Environ Saf 148:1054–1066

Aydinalp C, FitzPatrick EA, Cresser MS (2005) Heavy metal pollution in some soil and water resources of Bursa Province, Turkey. Commun Soil Sci Plant Anal 36(13–14):1691–1716

Babich H, Stotzky G (1985) Heavy metal toxicity to microbe-mediated ecologic processes: a review and potential application to regulatory policies. Environ Res 36(1):111–137

Baluja G, Gonzalez J, Rico C, Hernandez LM (1985) Sources and transport of organochlorine compounds and heavy metals into waters of the National Park of Donana. Bull Environ Contam Toxicol 35(4):482–489

Banzi FP, Msaki PK, Mohammed NK (2015) Assessment of heavy metal concentration in water around the proposed Mkuju River Uranium Project in Tanzania. Tanzan J Sci 41(1):8–18

Barlas N, Akbulut N, Aydoğan M (2005) Assessment of heavy metal residues in the sediment and water samples of Uluabat Lake, Turkey. Bull Environ Contam Toxicol 74(2):286–293

Bhuiyan MAH, Suruvi NI, Dampare SB, Islam M, Quraishi SB, Ganyaglo S, Suzuki S (2011) Investigation of the possible sources of heavy metal contamination in lagoon and canal water in the tannery industrial area in Dhaka, Bangladesh. Environ Monit Assess 175(1–4):633–649

Blake WH, Walsh RP, Reed JM, Barnsley MJ, Smith J (2007) Impacts of landscape remediation on the heavy metal pollution dynamics of a lake surrounded by non-ferrous smelter waste. Environ Pollut 148(1):268–280

Bonanno G, Giudice RL (2010) Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. Ecol Indic 10 (3):639–645

Bradley S, Woods WL (1997) Cd, Cr, Cu, Ni and Pb in the water column and sediments of the Ob-Irtysh Rivers, Russia. Mar Pollut Bull 35(7–12):270–279

Bubb JM, Lester JN (1994) Anthropogenic heavy metal inputs to lowland river systems, a case study. The River Stour, UK. Water Air Soil Pollut 78(3–4):279–296

CEC (Council of the European Communities) (1991) Council Directive of 21 May 1991 concerning urban waste water treatment (91/271/EEC). Off J Eur Communities 135:40–52

Cenci R, Martin J (2004) Concentration and fate of trace metals in Mekong River Delta. Sci Total Environ 332(1–3):167–182

Chen X, Zhang S (1986) A study on chemical speciation and distribution characteristics of heavy metals in Xiangjiang River water. Acta Sci Circumst 6(2):131–140. (in Chinese)

Cheng P, Li X (2017) Heavy metals pollution and related health risk assessment of Yang River: spatial and temporal variation. Chin J Environ Eng 11(8):4513–4519. (in Chinese)

Cheng C, Chen ZL, Bi CJ, Zhang C, Shi GT, Yang J (2009) Accumulate characteristics of lead, cadmium in drinking water sources of Huangpu River, Shanghai. Resour Environ Yangtze Basin 18(10):948–953. (in Chinese)

Cui B, Zhang Q, Zhang K, Liu X, Zhang H (2011) Analyzing trophic transfer of heavy metals for food webs in the newly-formed wetlands of the Yellow River Delta, China. Environ Pollut 159 (5):1297–1306

Dai MH, Martin JM (1995) First data on trace metal level and behaviour in two major Arctic river-estuarine systems (Ob and Yenisey) and in the adjacent Kara Sea, Russia. Earth Planet Sci Lett 131(3–4):127–141

de Miguel E, Iribarren I, Chacon E, Ordonez A, Charlesworth S (2007) Risk-based evaluation of the exposure of children to trace elements in playgrounds in Madrid (Spain). Chemosphere 66 (3):505–513

DeLeon IR, Byrne CJ, Peuler EA, Antoine SR, Schaeffer J, Murphy RC (1986) Trace organic and heavy metal pollutants in the Mississippi River. Chemosphere 15(6):795–805

Demirak A, Yilmaz F, Tuna AL, Ozdemir N (2006) Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. Chemosphere 63(9):1451–1458

Deng J, Gu M, Chen M, Feng X (2016) Investigation of pollutants in Guangzhou river and their correlation with the heavy metals. J Zhongkai Univ Agric Eng 29(4):38–43. (in Chinese)

Dorten WS, Elbaz-Poulichet F, Mart LR, Martin JM (1991) Reassessment of the river input of trace metals into the Mediterranean Sea. Ambio 20:2–6

Duan J, Tan J (2013) Atmospheric heavy metals and arsenic in China: situation, sources and control policies. Atmos Environ 74:93–101

Dupré B, Gaillardet J, Rousseau D, Allègre CJ (1996) Major and trace elements of river-borne material: the Congo Basin. Geochim Cosmochim Acta 60(8):1301–1321

Ebrahimpour M, Mushrifah I (2008) Heavy metal concentrations in water and sediments in Tasik Chini, a freshwater lake, Malaysia. Environ Monit Assess 141(1–3):297–307
Elbaz-Poulichet F, Martin J, Huang W, Zhu J (1987) Dissolved Cd behaviour in some selected French and Chinese estuaries. Consequences on Cd supply to the ocean. Mar Chem 22 (2–4):125–136
Elbaz-Poulichet F, Garnier JM, Guan DM, Martin JM, Thomas AJ (1996) The conservative behaviour of trace metals (Cd, Cu, Ni and Pb) and As in the surface plume of stratified estuaries: example of the Rhône River (France). Estuar Coast Shelf Sci 42(3):289–310
Facchinelli A, Sacchi E, Mallen L (2001) Multivariate statistical and GIS-based approach to identify heavy metal sources in soils. Environ Pollut 114(3):313–324
Fan Q, He J, Xue H, Lü C, Sun Y, Shen L, Liang Y, Bai S (2008) Heavy metal pollution in the Baotou section of the Yellow River, China. Chem Speciat Bioavailab 20(2):65–76
Farkas A, Salanki J, Varanka I (2000) Heavy metal concentrations in fish of Lake Balaton. Lakes Reserv Res Manag 5(4):271–279
Faton M, Amelina A, Albert K, Adrian D, Jamarber M, Ferdi B, Sulejman S (2015) Water and sediment heavy metal pollution in Ereniku River of Kosovo. Albanian J Agric Sci 14 (2):137–148
Gao Q, Li Y, Cheng Q, Yu M, Hu B, Wang Z, Yu Z (2016) Analysis and assessment of the nutrients, biochemical indexes and heavy metals in the Three Gorges Reservoir, China, from 2008 to 2013. Water Res 92:262–274
Gong X (2011) The characteristics of eco-geochemistry and heavy metals pollution in Fenhe River in Shanxi Province. Central South University, Changsha. (in Chinese)
Guay CK, Zhulidov AV, Robarts RD, Zhulidov DA, Gurtovaya TY, Holmes RM, Headley JV (2010) Measurements of Cd, Cu, Pb and Zn in the lower reaches of major Eurasian arctic rivers using trace metal clean techniques. Environ Pollut 158(2):624–630
Guieu C, Huang WW, Martin JM, Yong YY (1996) Outflow of trace metals into the Laptev Sea by the Lena River. Mar Chem 53(3–4):255–267
Guieu C, Martin JM, Tankere S, Mousty F, Trincherini P, Bazot M, Dai M (1998) On trace metal geochemistry in the Danube River and western Black Sea. Estuar Coast Shelf Sci 47(4):471–485
Gümgüm B, Tez Z, Gülsün Z (1994) Heavy metal pollution in water, sediment and fish from the Tigris River in Turkey. Chemosphere 29(1):111–116
Hatje V, Bidone E, Maddock J (1998) Estimation of the natural and anthropogenic components of heavy metal fluxes in fresh water Sinos River, Rio Grande do Sul State, South Brazil. Environ Technol 19(5):483–487
Hu C, Deng ZM, Xie YH, Chen XS, Li F (2015) The risk assessment of sediment heavy metal pollution in the East Dongting Lake Wetland. J Chem 2015:835487
Huang W, Martin J, Seyler P, Zhang J, Zhong X (1988) Distribution and behaviour of arsenic in the Huang He (Yellow River) estuary and Bohai Sea. Mar Chem 25(1):75–91
Huang Y, Li T, Wu C, He Z, Japenga J, Deng M, Yang X (2015) An integrated approach to assess heavy metal source apportionment in peri-urban agricultural soils. J Hazard Mater 299:540–549
Ismail Z, Salim K, Othman SZ, Ramli AH, Shirazi SM, Karim R, Khoo SY (2013) Determining and comparing the levels of heavy metal concentrations in two selected urban river water. Measurement 46(10):4135–4144
Järup L (2003) Hazards of heavy metal contamination. Br Med Bull 68:167–182
Jiang X, Wang W, Wang S, Zhang B, Hu J (2012) Initial identification of heavy metals contamination in Taihu Lake, a eutrophic lake in China. J Environ Sci 24(9):1539–1548
Kar D, Sur P, Mandai S, Saha T, Kole R (2008) Assessment of heavy metal pollution in surface water. Int J Environ Sci Technol 5(1):119–124
Karadede H, Ünlü E (2000) Concentrations of some heavy metals in water, sediment and fish species from the Atatürk Dam Lake (Euphrates), Turkey. Chemosphere 41(9):1371–1376
Karadede-Akin H, Ünlü E (2007) Heavy metal concentrations in water, sediment, fish and some benthic organisms from Tigris River, Turkey. Environ Monit Assess 131(1–3):323–337

Karbassi A, Monavari S, Bidhendi GRN, Nouri J, Nematpour K (2008) Metal pollution assessment of sediment and water in the Shur River. Environ Monit Assess 147(1–3):107
Kelessidis A, Stasinakis AS (2012) Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag 32(6):1186–1195
Kendall MG (1975) Rank correlation methods, 4th edn. Charles Griffin, London
Khan R, Israili S, Ahmad H, Mohan A (2005) Heavy metal pollution assessment in surface water bodies and its suitability for irrigation around the Neyevli lignite mines and associated industrial complex, Tamil Nadu, India. Mine Water Environ 24(3):155–161
Kim Y, Kim BK, Kim K (2010) Distribution and speciation of heavy metals and their sources in Kumho River sediment, Korea. Environ Earth Sci 60(5):943–952
Kimball BA, Callender E, Axtmann EV (1995) Effects of colloids on metal transport in a river receiving acid mine drainage, upper Arkansas River, Colorado, USA. Appl Geochem 10 (3):285–306
Kisi O, Ay M (2014) Comparison of Mann-Kendall and innovative trend method for water quality parameters of the Kizilirmak River, Turkey. J Hydrol 513:362–375
Konhauser K, Powell M, Fyfe W, Longstaffe F, Tripathy S (1997) Trace element chemistry of major rivers in Orissa State, India. Environ Geol 29(1–2):132–141
Krika A, Krika F (2017) Evaluation of the status of heavy metal pollution in surface water and sediments of the Nil River (North Eastern Algeria). Pollution 3(2):301–310
Kumar RN, Solanki R, Kumar JN (2013) Seasonal variation in heavy metal contamination in water and sediments of river Sabarmati and Kharicut canal at Ahmedabad, Gujarat. Environ Monit Assess 185(1):359–368
Lalah J, Ochieng E, Wandiga S (2008) Sources of heavy metal input into Winam Gulf, Kenya. Bull Environ Contam Toxicol 81(3):277–284
Larsen RK, Baker JE (2003) Source apportionment of polycyclic aromatic hydrocarbons in the urban atmosphere: a comparison of three methods. Environ Sci Technol 37(9):1873–1881
Li X (2009) Studies on distribution characteristics of copper in river and soil around Dahong Shan copper mine, Yunnan Province. Capital Normal University, Beijing. (in Chinese)
Li YL, Liu JL (2009) Health risk assessment on heavy metal pollution in the water environment of Luan River. J Agro-Environ Sci 28(6):1177–1184. (in Chinese)
Li S, Zhang Q (2010) Risk assessment and seasonal variations of dissolved trace elements and heavy metals in the Upper Han River, China. J Hazard Mater 181(1–3):1051–1058
Li RP, Wang AJ, Cao DH, Wang GS, Li YK, Geng N (2008) Geochemical characteristics of heavy metals in water bodies and sediments of the Bijiang River drainage area, western Yunnan, China. Geol Bull China 27(7):1071–1078. (in Chinese)
Li M, Liu QJ, Zhou WB (2010) Assessment on health risks associated with heavy metal pollutants for water environment of watersheds of Poyang Lake. J Anhui Agric Sci 38(32):18278–18280. (in Chinese)
Li FS, Lu Y, Li CX, Huang WX, Zhou LL, Wang SY, Zhang Q, Yao JB, Zuo Y (2013) The distribution, source analysis and comprehensive evaluation in urban lakes of contaminants from Anqing City. J Anqing Teachers College (Nat Sci Ed) 19(4):98–103. (in Chinese)
Liu ZT, Li CH, Zhang GY (2010) Application of principal component analysis to the distributions of heavy metals in the water of lakes and reservoirs in Yunnan Province. Res Environ Sci 23 (4):459–466. (in Chinese)
Liu H, Zhang Z, Li W (2011) Assessment and spatial patterns of trace elements and heavy metals from water body and sediments in the Liangzi Lake. Resour Environ Yangtze Basin 20 (Z1):105–111. (in Chinese)
Liu X, Zhai Y, Zhu Y, Liu Y, Chen H, Li P, Peng C, Xu B, Li C, Zeng G (2015) Mass concentration and health risk assessment of heavy metals in size-segregated airborne particulate matter in Changsha. Sci Total Environ 517:215–221
Loska K, Wiechuła D (2003) Application of principal component analysis for the estimation of source of heavy metal contamination in surface sediments from the Rybnik Reservoir. Chemosphere 51:723–733

Lu J, Zhong X, Wu H, Wang H (2016) Vertical distribution characteristics of heavy metals in lake under different hydrodynamic conditions. Trans Chin Soc Agric Mach 47(2):179–184. (in Chinese)

Luo W (1984) Preliminary analysis of heavy metals pollution in the Zhujiang River (Pearl River) estuarine area. Mar Sci Bull 3(5):64–69. (in Chinese)

Magdaleno A, de Cabo L, Arreghini S, Salinas C (2014) Assessment of heavy metal contamination and water quality in an urban river from Argentina. Braz J Aquat Sci Technol 18(1):113–120

Malm O, Pfeiffer W, Fiszman M, Azcue J (1988) Transport and availability of heavy metals in the Paraíba do Sul-Guandu River system, Rio de Janeiro state, Brazil. Sci Total Environ 75 (2–3):201–209

Mann HB (1945) Nonparametric tests against trend. Econometrica 13:245–259

Mao X, Chen X, Li CA, Zhang YF (2013) Distribution of heavy metal elements in surface water from three lakes in Daye City. Saf Environ Eng 20(5):33–37. (in Chinese)

Martin J, Guan D, Elbaz-Poulichet F, Thomas A, Gordeev V (1993) Preliminary assessment of the distributions of some trace elements (As, Cd, Cu, Fe, Ni, Pb and Zn) in a pristine aquatic environment: the Lena River estuary (Russia). Mar Chem 43(1–4):185–199

Martin JM, Huang WW, Yoon YY (1994) Level and fate of trace metals in the lagoon of Venice (Italy). Mar Chem 46(4):371–386

McFarlane G, Franzin W (1978) Elevated heavy metals: a stress on a population of white suckers, *Catostomus commersoni*, in Hamell Lake, Saskatchewan. J Fish Board Can 35(7):963–970

Meng X (2016) Distributions, historical trends and potential risk assessment of heavy metals in Dianchi Lake, China. Kunming University of Science and Technology, Kunming. (in Chinese)

Miller J, Hudson-Edwards K, Lechler P, Preston D, Macklin M (2004) Heavy metal contamination of water, soil and produce within riverine communities of the Rıo Pilcomayo basin, Bolivia. Sci Total Environ 320(2–3):189–209

Moore F, Forghani G, Qishlaqi A (2009) Assessment of heavy metal contamination in water and surface sediments of the Maharlu Saline Lake, SW Iran. Iran J Sci Technol 33(1):43–55

Mortvedt JJ (1996) Heavy metal contaminants in inorganic and organic fertilizers. Fertil Environ Dev Plant Soil Sci 66:55–61

Muhammad S, Shah MT, Khan S (2011) Health risk assessment of heavy metals and their source apportionment in drinking water of Kohistan Region, Northern Pakistan. Microchem J 98 (2):334–343

Müller G, Förstner U (1975) Heavy metals in sediments of the Rhine and Elbe estuaries: mobilization or mixing effect? Environ Geol 1(1):33–39

Munksgaard NC, Parry DL (2001) Trace metals, arsenic and lead isotopes in dissolved and particulate phases of North Australian coastal and estuarine seawater. Mar Chem 75(3):165–184

Muwanga A, Barifaijo E (2006) Impact of industrial activities on heavy metal loading and their physico-chemical effects on wetlands of Lake Victoria basin (Uganda). Afr J Sci Technol 7 (1):51–63

Nasehi F, Hassani A, Monavvari M, Karbassi A, Khorasani N, Imani A (2012) Heavy metal distributions in water of the Aras River, Ardabil, Iran. J Water Resour Protect 4:73–78

Nguyen H, Leermakers M, Osán J, Török S, Baeyens W (2005) Heavy metals in Lake Balaton: water column, suspended matter, sediment and biota. Sci Total Environ 340(1–3):213–230

Oyewale A, Musa I (2006) Pollution assessment of the lower basin of Lakes Kainji/Jebba, Nigeria: heavy metal status of the waters, sediments and fishes. Environ Geochem Health 28(3):273–281

Özmen H, Külahçı F, Çukurovalı A, Doğru M (2004) Concentrations of heavy metal and radioactivity in surface water and sediment of Hazar Lake (Elazığ, Turkey). Chemosphere 55 (3):401–408

Pathiratne A, Chandrasekera L, Pathiratne K (2009) Use of biomarkers in Nile tilapia (*Oreochromis niloticus*) to assess the impacts of pollution in Bolgoda Lake, an urban water body in Sri Lanka. Environ Monit Assess 156(1–4):361

Pettine M, Bianchi M, Martinotti W, Muntau H, Renoldi M, Tartari G (1996) Contribution of the Lambro River to the total pollutant transport in the Po watershed (Italy). Sci Total Environ 192 (3):275–297

Polprasert C (1982) Heavy metal pollution in the Chao Phraya River estuary, Thailand. Water Res 16(6):775–784

Presley B, Trefry J, Shokes R (1980) Heavy metal inputs to Mississippi delta sediments. Water Air Soil Pollut 13(4):481–494

Qin Y, Cao W, Ma Y, Zhang L, Liu Z, Chang X (2015) Distribution and standardized analysis of heavy metal among surface water, suspended solids and surface sediments in the Yangtze-Taihu water diversion section. Environ Pollut Control 37(6):5–10. (in Chinese)

Rahman MS, Saha N, Molla AH (2014) Potential ecological risk assessment of heavy metal contamination in sediment and water body around Dhaka export processing zone, Bangladesh. Environ Earth Sci 71(5):2293–2308

Rahman M, Ziku A, Choudhury TR, Ahmad J, Mottaleb M (2015) Heavy metals contaminations in irrigated vegetables, soils, river water: a compressive study Chilmari, Kurigram, Bangladesh. Int J Environ Ecol Fam Urban Stud 5(5):29–42

Ramos L, Fernández M, González M, Hernández L (1999) Heavy metal pollution in water, sediments, and earthworms from the Ebro River, Spain. Bull Environ Contam Toxicol 63 (3):305–311

Rashed M (2001) Monitoring of environmental heavy metals in fish from Nasser Lake. Environ Int 27(1):27–33

Reddy MV, Babu KS, Balaram V, Satyanarayanan M (2012) Assessment of the effects of municipal sewage, immersed idols and boating on the heavy metal and other elemental pollution of surface water of the eutrophic Hussainsagar Lake (Hyderabad, India). Environ Monit Assess 184 (4):1991–2000

Ren J, Williams PN, Luo J, Ma H, Wang X (2015) Sediment metal bioavailability in Lake Taihu, China: evaluation of sequential extraction, DGT, and PBET techniques. Environ Sci Pollut Res 22(17):12919–12928

Reza R, Singh G (2010) Heavy metal contamination and its indexing approach for river water. Int J Environ Sci Technol 7(4):785–792

Salati S, Moore F (2010) Assessment of heavy metal concentration in the Khoshk River water and sediment, Shiraz, Southwest Iran. Environ Monit Assess 164(1–4):677–689

Say P, Harding J, Whitton B (1981) Aquatic mosses as monitors of heavy metal contamination in the River Etherow, Great Britain. Environ Pollut B 2(4):295–307

Schierup HH, Larsen VJ (1981) Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. I. Availability, uptake and translocation of heavy metals in *Phragmites australis* (Cav.) Trin. Aquat Bot 11:197–210

Sharley DJ, Sharp SM, Bourgues S, Pettigrove VJ (2016) Detecting long-term temporal trends in sediment-bound trace metals from urbanised catchments. Environ Pollut 219:705–713

Sharma R, Vaishnav V (2015) Assessment of heavy metal toxicity in surface water of river Bandi, Pali, Rajasthan. Adv Appl Phys Chem Sci A Sustain Approach 164–169

Shen Z, Dong W, Zhang L, Chen X (1989) Geochemical characteristics of heavy metals in the Xiangjiang River, China. Hydrobiologia 176(1):253–262

Shephard BK, McIntosh AW, Atchison GJ, Nelson DW (1980) Aspects of the aquatic chemistry of cadmium and zinc in a heavy metal contaminated lake. Water Res 14(8):1061–1066

Shi X (2014) The time and space distribution and geochemical characteristics of heavy metals in Haihe River trunk stream water. Tianjin Normal University, Tianjin. (in Chinese)

Shiller AM, Boyle EA (1987) Variability of dissolved trace metals in the Mississippi River. Geochim Cosmochim Acta 51(12):3273–3277

Sin YM, Wong MK, Chou LM, Alias NB (1991) A study of the heavy metal concentrations of the Singapore River. In: Fourth symposium on our environment, Dordrecht, pp 481–494

Singare P, Lokhande R, Naik K (2010) A case study of some lakes located at and around thane city of Maharashtra, India, with special reference to physico-chemical properties and heavy metal content of lake water. Interdiscip Environ Rev 11(1):90–107
Singare P, Talpade M, Dagli D, Bhawe V (2013) Heavy metal content in water of Bhavan's College Lake of Andheri, Mumbai. Int Lett Chem Phys Astron 8(2):94–104
Singh O, Labana S, Pandey G, Budhiraja R, Jain R (2003) Phytoremediation: an overview of metallic ion decontamination from soil. Appl Microbiol Biotechnol 61(5–6):405–412
Smolders A, Lock R, Van der Velde G, Hoyos RM, Roelofs J (2003) Effects of mining activities on heavy metal concentrations in water, sediment, and macroinvertebrates in different reaches of the Pilcomayo River, South America. Arch Environ Contam Toxicol 44(3):0314–0323
Stoica AI (1999) Analytical studies on the pollution of Arges River. Crit Rev Anal Chem 29 (3):243–247
Su W, Liu JS, Li F (2006) Assessment on health risk of heavy metals in the second Songhua River. J Agro-Environ Sci 25(6):1611–1615. (in Chinese)
Sun QZ, Zang SY (2012) Pollution evaluation and forecast of heavy mental in Lake of Zhalong Wetland, China. J Agro-Environ Sci 31(11):2242–2248. (in Chinese)
Sun C, Chen ZL, Zhang C, Shi GT, Bi CJ (2009) Health risk assessment of heavy metals in drinking water sources in Shanghai, China. Res Environ Sci 22(1):60–65. (in Chinese)
Sundaray SK (2010) Application of multivariate statistical techniques in hydrogeochemical studies-a case study: Brahmani-Koel River (India). Environ Monit Assess 164(1–4):297–310
Tao Y, Yuan Z, Xiaona H, Wei M (2012) Distribution and bioaccumulation of heavy metals in aquatic organisms of different trophic levels and potential health risk assessment from Taihu lake, China. Ecotoxicol Environ Saf 81:55–64
Thorp VJ, Lake PS (1973) Pollution of a Tasmanian river by mine effluents. II. Distribution of macroinvertebrates. Int Rev Gesamten Hydrobiol Hydrograph 58(6):885–892
Tian LF, Hu JW, Qin FX, Huang XF, Wu D, Duan SM (2011) Statistical analysis of distribution characteristics of heavy metals in surface water from Baihua Lake. Res Environ Sci 24 (3):259–267
Turgut C (2003) The contamination with organochlorine pesticides and heavy metals in surface water in Küçük Menderes River in Turkey, 2000–2002. Environ Int 29(1):29–32
Varol M (2013) Dissolved heavy metal concentrations of the Kralkızı, Dicle and Batman dam reservoirs in the Tigris River basin, Turkey. Chemosphere 93(6):954–962
Varol M, Şen B (2012) Assessment of nutrient and heavy metal contamination in surface water and sediments of the upper Tigris River, Turkey. Catena 92:1–10
Varol M, Gökot B, Bekleyen A (2010) Assesment of water pollution in the Tigris River in Diyarbakır, Turkey. Water Pract Technol 5(1):1–13
Waara KO (1992) Effects of copper, cadmium, lead and zinc on nitrate reduction in a synthetic water medium and lake water from Northern Sweden. Water Res 26(3):355–364
Wan J, Yan W, Xie T (2007) Research on heavy metals pollution status of Lean River, Lake Poyang basin. J Lake Sci 19(4):421–427. (in Chinese)
Wang W, Liu XD, Zhao LW, Guo DF, Lu YQ (2003) Assessment of the phase-out of leaded gasoline in Tianjin, China using isotope technique. China Environ Sci 23(6):627–630. (in Chinese)
Wang L, Wang Y, Xu C, An Z, Wang S (2011) Analysis and evaluation of the source of heavy metals in water of the River Changjiang. Environ Monit Assess 173(1–4):301–313
Wang LM, Zhang S, Zhao SN, Wu Y (2014a) Spatial distribution characteristics of heavy metals in Ulansuhai Lake. J Environ Health 31(12):1088–1089. (in Chinese)
Wang Z, Yao L, Liu G, Liu W (2014b) Heavy metals in water, sediments and submerged macrophytes in ponds around the Dianchi Lake, China. Ecotoxicol Environ Saf 107:200–206
Wang H, Sun L, Liu Z, Luo Q, Wu H, Wang X (2015) Study on the character of health risk in the water environment of Hun River. Asian J Ecotoxicol 10(2):394–402. (in Chinese)
Wang YH, Liu YX, Liu HH, Liu XH, Lu ZK, Li J (2018) Chemical composition and heavy metal distribution in surface water of typical inland rivers in Qinghai. Chin J Ecol 37(3):734–742. (in Chinese)

Winner R, Boesel M, Farrell M (1980) Insect community structure as an index of heavy-metal pollution in lotic ecosystems. Can J Fish Aquat Sci 37(4):647–655
Wu P, Liu CQ, Zhang GP, Yang YG (2002) Characteristics of heavy metal pollution in stream of zinc smelting area, northwest of Guizhou. Agro-Environ Protect 21(5):443–446. (in Chinese)
Wu L, Liu GJ, Zhou CC, Liu RQ (2018) Temporal-spatial distribution and pollution assessment of dissolved heavy metals in Chaohu Lake. Environ Sci 39(2):738–747. (in Chinese)
Wuana RA, Okieimen FE (2011) Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation. Isrn Ecol 2011:402647. https://doi.org/10.5402/2011/402647
Yan L, Sun M, Yao X, Gong N, Li X, Qi M (2018) Lake water in the Tibet Plateau: quality change and current status evaluation. Acta Sci Circumst 38(3):900–910. (in Chinese)
Yang ZF, Xia XQ, Yu T, Hou QY, Cao TN, Zhong J (2008) Distribution and fluxes of As and trace metals in the Dongting Lake water system, Hunan Province, China. Geoscience 22(6):897–908. (in Chinese)
Yang Y, Christakos G, Guo M, Xiao L, Huang W (2017) Space-time quantitative source apportionment of soil heavy metal concentration increments. Environ Pollut 223:560–566
Yi Y, Yang Z, Zhang S (2011) Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. Environ Pollut 159(10):2575–2585
Yousef Y, Wanielista M, Hvitved-Jacobsen T, Harper H (1984) Fate of heavy metals in stormwater runoff from highway bridges. Sci Total Environ 33(1–4):233–244
Yue R, Zhang H, Shi R, Wang MP (2015) Research on statistical distribution characteristics of heavy metals in Wuliangsuhai Lake. J Anhui Agric Sci 43(31):376–379. (in Chinese)
Zeng QR, Yang RB, Tie BQ, Zhou XH, Liao MC, Wang XC (2002) Control of pollution of heavy metals on farmland by ecological engineering in wast and west valley in Chen County. Agro-Environ Protect 21(5):428–431. (in Chinese)
Zhang J (2013) The research on distribution characteristics of heavy metals and agricultural application in water and sediment of Jinyang Lake. Shanxi University, Taiyuan. (in Chinese)
Zhang XJ, Hu MA (2006) Heavy metal distribution of creek water and its sediments in Tieshan, Daye, Hubei Province. Geol Sci Technol Inform 25(2):89–92. (in Chinese)
Zhang X, Wang K, Hua J, Zhai Z, Zhu X, Jiang H, Chu L (2016) The distribution of heavy metals in Jianshan River of Jianshan River watershed. J Southwest For Univ 36(2):42–48. (in Chinese)
Zilkir HM, Sharmin S, Shikazonol N (2006) Heavy metal pollution assessment in water and sediments of Turag River at Tongi area in Bangladesh. Int J Lakes Riv 1(1):85–96
Zingde M, Rokade M, Mandalia A (1988) Heavy metals in Mindhola river estuary, India. Mar Pollut Bull 19(10):538–540
Zwolsman JJ, van Eck GT (1999) Geochemistry of major elements and trace metals in suspended matter of the Scheldt estuary, southwest Netherlands. Mar Chem 66(1–2):91–111

Anaerobic Microbial Degradation of Polycyclic Aromatic Hydrocarbons: A Comprehensive Review

Kartik Dhar, Suresh R. Subashchandrabose, Kadiyala Venkateswarlu, Kannan Krishnan, and Mallavarapu Megharaj

Contents

K. Dhar
Global Centre for Environmental Remediation (GCER), Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia

Department of Microbiology, University of Chittagong, Chittagong, Bangladesh
e-mail: kartik.dhar@uon.edu.au

S. R. Subashchandrabose · K. Krishnan · M. Megharaj (✉)
Global Centre for Environmental Remediation (GCER), Faculty of Science, The University of Newcastle, Callaghan, NSW, Australia
e-mail: suresh.subashchandrabose@newcastle.edu.au; kannan.krishnan@newcastle.edu.au; megh.mallavarapu@newcastle.edu.au

K. Venkateswarlu
Formerly Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, India
e-mail: venkateswarlu.kadiyala@gmail.com

P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology, Volume 251*, Reviews of Environmental Contamination and Toxicology Volume 251,
https://doi.org/10.1007/398_2019_29

Abbreviations

$\Delta G^{\circ\prime}$	Standard Gibbs free energy change
1,2,3,4-THNA	1,2,3,4-Tetrahydro-2-naphthoic acid
1-MN	1-Methylnaphthalene
1-NA	1-Naphthoic acid
2-DMNA	2-Dimethylnaphthalene
2-MN	2-Methylnaphthalene
2-NA	2-Naphthoic acid
5,6,7,8-THNA	5,6,7,8-Tetrahydro-2-naphthoic acid
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BaP	Benzo(a)pyrene
Bcr	Benzoyl-CoA reductase
BESA	Bromoethane sulfonic acid
Bns	Beta-oxidation of naphthyl-2-methylsuccinate
Bss	Benzylsuccinate synthase
BTEX	Benzene, toluene, ethylbenzene, and xylene
CoA	Coenzyme A
DO	Dissolved oxygen
$E^{0\prime}$	Standard reduction potential
FBR	Fluidized bed reactor
GC	Gas chromatography
H_2O_2	Hydrogen peroxide
HH-2-NA	Hexahydro-2-naphthoic acid
HMW	High molecular weight
kDa	Kilodalton
LC	Liquid chromatography
LC-ESI-MS-MS	Liquid chromatography electrospray ionization tandem mass spectrometry

LiP	Lignin peroxidase
LMW	Low molecular weight
$\log K_{OW}$	Octanol-water partition coefficient
MGP	Manufactured gas plant sites
MNA	Methylnaphthoic acid
MnP	Manganese-dependent peroxidase
MS	Mass spectrometry
Ncr	Naphthoyl-CoA reductase
NMeS	Naphthyl-2-methylenesuccinic acid
Nms	2-Napthylmethylsuccinate synthase
NMS	Naphthyl-2-methylsuccinic acid
NRB	Nitrate-reducing bacteria
OYE	Old yellow enzyme
PAHs	Polycyclic aromatic hydrocarbons
POP	Persistent organic pollutants
PpcA	Phenylphosphate carboxylase
Q-TOF-MS	Quadrupole time-of-flight mass spectrometry
rRNA	Ribosomal RNA
SOM	Soil organic matter
SRB	Sulfate-reducing bacteria
TCA	Tricarboxylic acid
TEA	Terminal electron acceptor
THNA	Tetrahydronaphthoic acid
TOC	Total organic carbon
T-RFLP	Terminal restriction fragment length polymorphism
UbiD	3-Polyprenyl-4-hydroxybenzoate decarboxylase
US EPA	United States Environmental Protection Agency

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of hundreds of related organic aromatic compounds consisting of at least two (e.g., naphthalene) or more (e.g., anthracene) fused benzene rings arranged in linear (e.g., naphthalene, anthracene), angular [e.g., dibenzo(a,h)anthracene], or cluster (e.g., pyrene) fashion. PAHs that are composed of only fused benzene rings are classified as alternant PAHs (Smith and March 2007). In addition to the regular hexagonal benzene ring, non-alternant PAHs contain an additional annealed cyclic structure, for example, a tetragonal or a pentagonal ring. Thus, naphthalene, phenanthrene, and pyrene are alternant PAHs, while fluorene, fluoranthene, cyclopenta(d,e,j)phenanthrene, 7H-benzanthrene, and indeno(1,2,3-c,d)pyrene are non-alternant PAHs (Blumer 1976; Maliszewska-Kordybach 1999; Neilson 2013; Abdel-Shafy and Mansour 2016). Two- and three-ring PAHs are generally regarded as low-molecular-weight (LMW) PAHs,

whereas those having four or more rings are considered as high-molecular-weight (HMW) PAHs. Incomplete combustion of carbonaceous materials and crude oil spills are the two major sources of PAHs in the environment (Maliszewska-Kordybach 1999; Lundstedt et al. 2007; Ohura 2007). They are ubiquitous environmental pollutants; many of them show toxic, mutagenic, and carcinogenic properties (White and Claxton 2004). Their complex and rigid aromatic structure, high resonance energy, and limited bioavailability make them chemically stable as well as resistant to microbial degradation. Due to the ubiquity, persistence, bioaccumulative tendency, and acute toxicity to biota, PAHs are regarded as a class of hazardous organic contaminants.

The United States Environmental Protection Agency (US EPA) announced 16 PAHs as "priority pollutants" in aquatic and terrestrial ecosystems (US EPA 1982). The US EPA (2008) extended the list of hazardous PAHs in January 2008 (see Fig. 1). Although PAHs are removed from the environment by physicochemical processes, biodegradation of the pollutants by bacteria, fungi, algae, and plants is regarded as the principal mechanism of detoxification and removal (Samanta et al. 2002). Aerobic degradation of PAHs is well studied, and associated biochemical mechanisms have already been elucidated. Aerobic bacterial degradation of PAHs initiates with the dioxygenase enzyme-catalyzed introduction of both atoms of molecular oxygen into the aromatic nucleus of PAHs (Cerniglia 1992; Kanaly and Harayama 2000; Haritash and Kaushik 2009). PAHs can dissipate from their sources to many environmental compartments where the oxygen level is too low or zero. Such an anaerobic environment exists in many habitats such as subsurface soil, groundwater, aquifer sediment, freshwater sediment, marine sediment, sewage sludge, anaerobic wastewater treatment plant, etc. Biodegradation of PAHs in an anaerobic environment is challenging because molecular oxygen that is involved in the first step of the degradation pathway is not available to serve as the terminal electron acceptor during aerobic respiration and as a substrate for dioxygenases. For many years, PAHs were thought to be refractory to anaerobic microbial degradation; unavailability of oxygen and lower energy yield in the anaerobic metabolism were believed to be the critical restraining factors. However, many facultative and strictly anaerobic bacteria and archaea are now known to degrade PAHs using alternative electron acceptors such as nitrate, iron(III), and sulfate. Furthermore, pathways of anaerobic naphthalene and 2-methylnaphthalene degradation in sulfate-reducing bacteria have been elucidated.

The understanding in the field of anaerobic biodegradation of PAHs is expanding consistently. Therefore, a critical appraisal of the field will help researchers to keep abreast of trends and state of the art of the knowledge. Only a few reviews in this field are available; most of them discussed PAHs degradation in association with monoaromatic compounds like benzene and toluene (Meckenstock et al. 2004, 2016; Foght 2008; Meckenstock and Mouttaki 2011). That too, many of the reviews focused primarily on degradation and metabolism of PAHs by sulfate-reducing bacteria (SRB). As such, only limited information is available on the facultative anaerobic degradation of PAHs by nitrate reducers, iron reducers, and methanogens. Therefore, the present review aims at providing a comprehensive critique of the

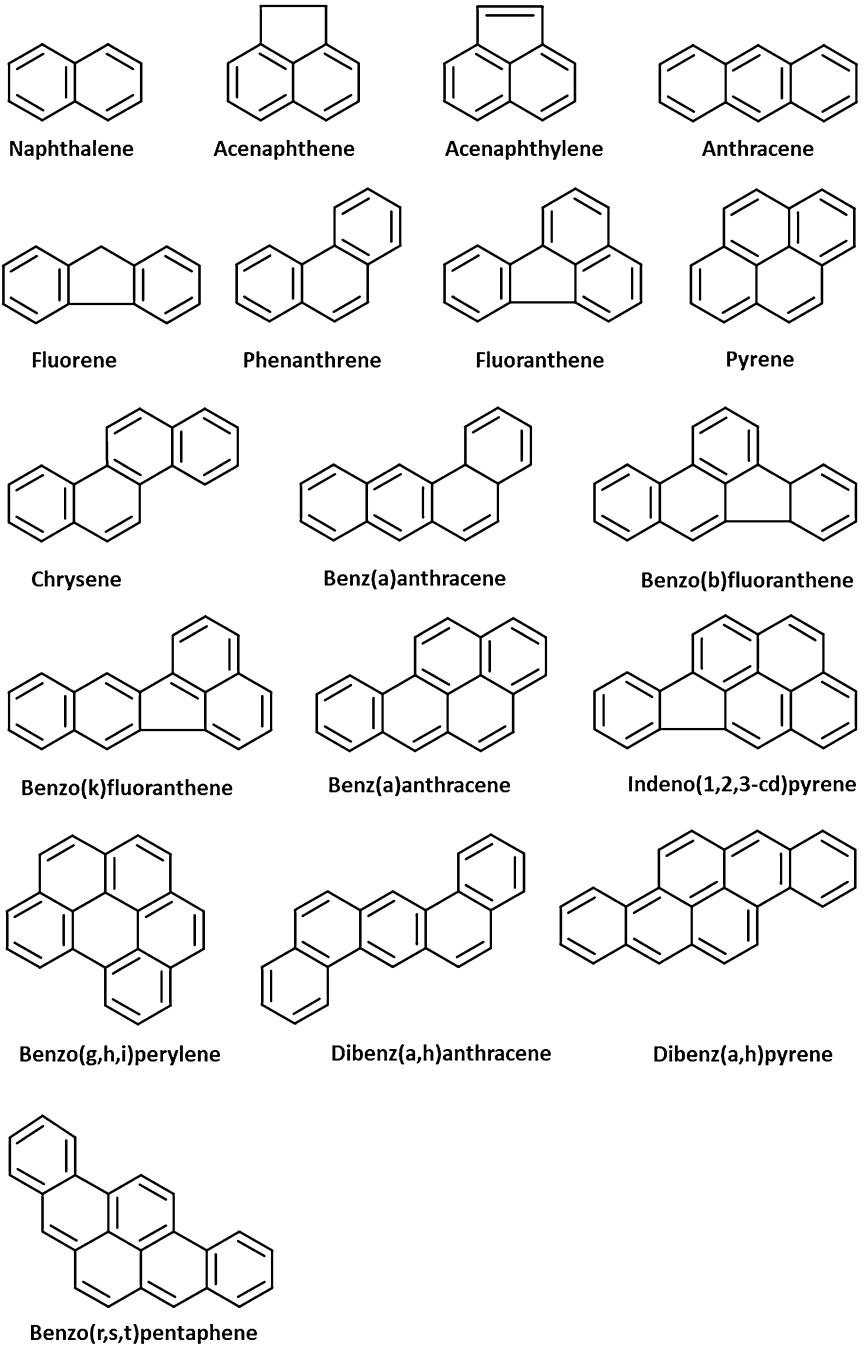

Fig. 1 Chemical structure of some PAHs of environmental concern

various aspects of anaerobic PAHs biodegradation. Initial few sections deal with the physicochemical properties, toxicity, sources, and possible fates of PAHs in the environment. The main discussion begins with an explanation of the process for development of anaerobic conditions and theoretical prediction of the thermodynamic feasibility of PAHs degradation under different reducing conditions. Later, PAHs degradation under various reducing conditions, their effects on anaerobic microbial community, and factors affecting anaerobic biodegradation are discussed. Furthermore, metabolic pathways of anaerobic PAHs degradation under various redox conditions and progress in the area of metabolic biomarker-based in situ degradation monitoring techniques are summarized. Finally, we identified some critical research gaps and suggested bioremediation approaches for mitigating PAHs contamination in anaerobic environments.

2 Physicochemical Properties of PAHs

The behavior, transport, and fate of PAHs in the environment largely depend on their physical and chemical properties. Three main aspects of PAHs as pollutants – bioavailability, persistence and bioaccumulation tendency – are strongly influenced by these physicochemical properties (Skupinska et al. 2004; Wiktorska et al. 2004; Abdel-Shafy and Mansour 2016). With an increase in molecular weight and number of benzene rings in the structure, their aqueous solubility decreases, resistance to oxidation and reduction increases, and vapor pressure drops. Common physical, chemical, and toxicological properties of some selected PAHs, those are listed in US EPA Toxic Release Inventory for polycyclic aromatic compounds, are listed in Table 1. Pure PAHs are white to pale yellow color solids. They are nonpolar, hydrophobic, and lipophilic. PAHs are slightly soluble in water, and, in general, their solubility in water decreases as the molecular weight increases (Table 1). They are soluble in many organic solvents and have a strong tendency to sorb to and accumulate in organic molecules of soil and sediments or in fat (Subashchandrabose et al. 2014). Water-soluble PAHs, such as naphthalene, have higher aqueous solubility and hence are more available for microbial degradation. HMW PAHs are less water soluble and thus less accessible for microbial attack and, therefore, remain persistent in the environment.

PAHs exist as a complex mixture in nature. The composition of a PAHs pool emitted from a combusted source depends on the properties of the combusting carbonaceous material and combustion temperature. Likewise, crude oil, petroleum fuels, coal tar, creosote, and asphalt contain different combinations of PAHs and their derivatives (Blumer 1976). Vapor pressure is an essential determinant of dispersion, transport, and fate of PAHs. LMW PAHs having higher vapor pressure are mostly emitted as gas phase in ambient air. HMW PAHs have lower vapor pressure, and they are released predominantly as particle form. Most of the time, the particles tend to be associated with the airborne particulates such as soot, dust, and fly ash. The octanol-water partition coefficient ($\log K_{OW}$) is also a crucial

Table 1 Physical, chemical, and toxicological properties of PAHs and PAHs derivatives

PAH		Formula	No. of rings	CAS Registry no.	Molecular weight (g mol^{-1})	Melt. point (°C)[a]	Aqueous solubility (mg L^{-1})	Vapor pressure (mm Hg at 25°C)	log K_{OW}[b]	Carcinogenicity		Mutagenicity
										IARC group	EPA	
LMW PAHs	Naphthalene	$C_{10}H_8$	2	91-20-3	128.174	80	31	0.085	3.3	2B	C	+
	Acenaphthene	$C_{12}H_{10}$	3	83-32-9	154.212	93	3.57–3.93	0.0022	3.92	3	D	NA
	Acenaphthylene	$C_{12}H_8$	3	208-96-8	152.196	89.4	3.93	0.0048	3.93	3	D	NA
	Anthracene	$C_{14}H_{10}$	3	120-12-7	178.234	216	1.29	6.56×10^{-6}	4.45	3	D	NA
	Fluorene	$C_{13}H_{10}$	3	86-73-7	166.218	114.8	1.69	6.0×10^{-4}	4.18	3	D	NA
	Phenanthrene	$C_{14}H_{10}$	3	85-01-8	178.234	99	1.1	1.21×10^{-4}	4.46	3	D	NA
HMW PAHs	Fluoranthene	$C_{16}H_{10}$	4	206-44-0	202.25	110.2	0.20–0.26	9.22×10^{-6}	5.16	3	D	NA
	Pyrene	$C_{16}H_{10}$	4	129-00-0	202.25	150.6	0.135	4.5×10^{-6}	4.88	3	D	NA
	Chrysene/benzo(a) phenanthrene	$C_{18}H_{12}$	4	218-01-9	228.294	255	2.0×10^{-3}	6.23×10^{-9}	5.73	3	B2	+
	7,12-Dimethylbenz(a) anthracene	$C_{20}H_{16}$	4	57-97-6	256.348	123	0.061	6.8×10^{-7}	5.8	NA	NA	+
	5-Methylchrysene	$C_{19}H_{14}$	4	3697-24-3	242.321	117.5	0.062	5.45×10^{-7}	6.07	2B	NA	NA
	Benzo(a)pyrene	$C_{20}H_{12}$	5	50-32-8	252.309	179	1.62×10^{-3}	5.49×10^{-9}	6.13	1	A2	NA
	Benzo(j)fluoranthene	$C_{20}H_{12}$	5	205-82-3	252.306	165.2	2.5×10^{-9}	2.7×10^{-8}	6.11	2B	NA	+
	Benz(a)anthracene	$C_{18}H_{12}$	5	56-55-3	228.294	155–157	9.4×10^{-3}	2.1×10^{-7}	5.76	2B	B2	+
	Benzo(b)fluoranthene	$C_{20}H_{12}$	5	205-99-2	252.309	168.4	0.0015	5.0×10^{-7}	5.78	2B	B2	+
	Benzo(k)fluoranthene	$C_{20}H_{12}$	5	207-08-9	252.316	217	0.00076	9.65×10^{-10}	6.11	2B	B2	+
	Dibenz(a,h) anthracene	$C_{22}H_{14}$	5	53-70-3	278.354	269	0.00166	9.55×10^{-10}	6.5	2A	B2	+
	Dibenz(a,h)acridine	$C_{21}H_{13}N$	5	226-36-8	279.342	228	0.159	7.51×10^{-10}	5.73	2B	NA	+
	Dibenz(a,j)acridine	$C_{21}H_{13}N$	5	224-42-0	279.342	216	0.159	1.05×10^{-9}	5.63	2B	NA	+
	7H-Dibenzo(c,g) carbazole	$C_{20}H_{13}N$	5	194-59-2	267.331	158	NA	3.4×10^{-9}	NA	2B	NA	NA
	3-Methylcholanthrene	$C_{21}H_{16}$	5	56-49-5	268.359	178	2.8×10^{-3}	4.3×10^{-8}	6.42	NA	NA	+

(continued)

Table 1 (continued)

PAH		Formula	No. of rings	CAS Registry no.	Molecular weight ($g\ mol^{-1}$)	Melt. point (°C)[a]	Aqueous solubility ($mg\ L^{-1}$)	Vapor pressure (mm Hg at 25°C)	log K_{OW}[b]	Carcinogenicity		Mutagenicity
										IARC group	EPA	
	Benzo(r,s,t) pentaphene	$C_{24}H_{14}$	6	189-55-9	302.376	283.6	7.4×10^{-5}	1.8×10^{-11}	7.28	2B	NA	+
	Dibenzo(a,e) fluoranthene	$C_{24}H_{14}$	6	5385-75-1	302.368	232	2.12×10^{-4}	7.33×10^{-11}	7.28	3	NA	+
	Dibenzo(a,e)pyrene	$C_{24}H_{14}$	6	192-65-4	302.368	244.4	1.6×10^{-4}	5.2×10^{-11}	7.28	3	NA	+
	Dibenzo(a,h)pyrene	$C_{24}H_{14}$	6	189-64-0	302.368	318	3.5×10^{-5}	6.4×10^{-12}	7.28	2B	NA	+
	Dibenzo(a,l)pyrene	$C_{24}H_{14}$	6	189-55-9	302.368	283.6	7.5×10^{-5}	2.17×10^{-8}	7.28	3	NA	NA
	Indeno(1,2,3-cd) pyrene	$C_{22}H_{12}$	6	193-39-5	276.338	164	6.2×10^{-2}	1.25×10^{-10}	6.7	2B	B2	+
	Benzo(g,h,i)perylene	$C_{22}H_{12}$	6	191-24-2	276.338	278.3	2.6×10^{-4}	1.0×10^{-10}	6.63	3	D	+

Sources: US EPA (1980, 2008), Miller et al. (1985), IARC (2010), O'Neil (2013), and Haynes (2014)

[a]Melting point

[b]Octanol-water coefficient

determinant of PAHs compartmentalization in the environment. It is a measure that expresses the extent of a substance to partition itself between an organic phase (*n*-octanol) and an aqueous phase. It is used for predicting the distribution of a substance in various environmental compartments, i.e., water, soil, sediment, and biota. A $\log K_{OW}$ value greater than four indicates that a chemical is likely to be sorbed strongly to minerals and organic matters (Karickhoff et al. 1979; Means et al. 1980). The $\log K_{OW}$ values for almost all the PAHs exceed four (Table 1). The values increase proportionally with the increase in molecular weight (Miller et al. 1985). Thus, benzo(a)pyrene (BaP) with a $\log K_{OW} = 6.13$ is expected to bind strongly to organic fraction of soil and sediment and to lipids of organisms. PAHs with high $\log K_{OW}$ may disappear quickly from aqueous mixture but remain persistent in soil and sediment. The propensity of PAHs to sorb to lipids indicates the potential of bioaccumulation in living organisms. Moreover, PAHs exhibit some notable characteristics like photosensitivity, conductivity, heat resistance, and corrosion resistance (Miller and Olejnik 2001; Northrop et al. 1956; Stein and Fahr 1985). Although PAHs are relatively stable compounds, they are prone to several chemical alteration processes in the natural environment. They may be subjected to photooxidation, chemical oxidation with oxidizing agents, hydroxylation, nitration, emulsification, as well as a range of microbial degradation processes. Some of the breakdown products are less toxic than the parental PAHs, while some nitro-, oxy-, amino-, and hydroxy-derivatives pose even greater toxicity (Nielsen et al. 1983; Neilson et al. 1998; Yu 2002; Kim et al. 2013; Neilson 2013; Andersson and Achten 2015).

3 Sources of PAHs

PAHs may not be abundant only in our planet, they are proposed to be widely distributed in the universe and constitute up to one fifth of all the carbon present in the galaxy (Allamandola et al. 1989; Cohen and Barlow 2005; Tielens 2005). They might have formed just after a couple of billion years after the Big Bang. The presence of anthracene and pyrene in Red Triangle nebula has been suggested based on the spectral signature analysis (Mulas et al. 2006). The new "PAHs world" hypothesis argues that the primordial soup might contain PAHs that in eons of time underwent several difficult changes and eventually transformed into the starting materials such as purines and pyrimidines for the origin of life (Peeters 2011). If the theory were proven right, it would shed some light on explaining the ubiquity of PAHs on Earth. PAHs are present in every sphere of Earth: atmosphere, hydrosphere, lithosphere, and biosphere. They can enter the environment in several ways. Based on their origin and mode of distribution, the sources of PAHs can be categorized into three major groups: pyrogenic, petrogenic, and diagenetic and biogenic (Fig. 2).

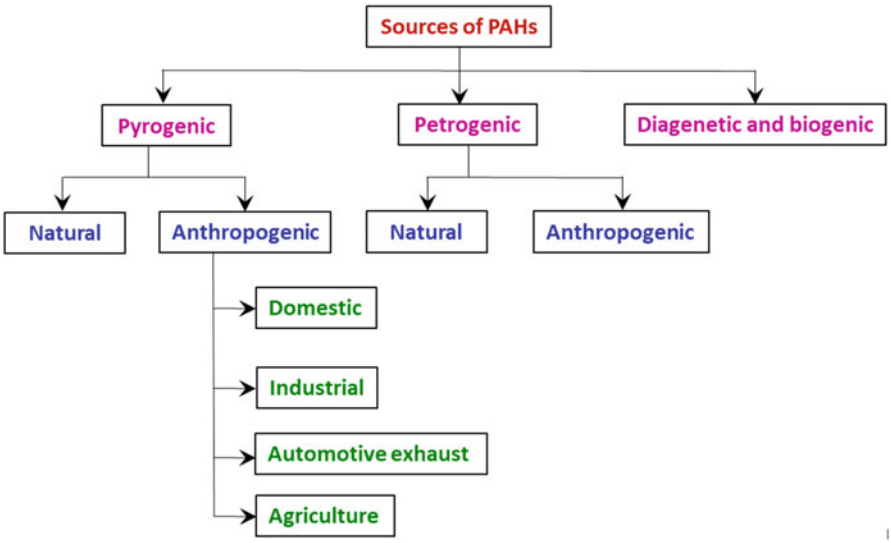

Fig. 2 Classification of PAHs sources based on their origin and mode of distribution

3.1 Pyrogenic Sources

Pyrolysis or thermal cracking is an irreversible thermochemical process in which organic matter decomposes at high temperature in the absence of oxygen. Incomplete combustion of fossil fuel and biomass during pyrolysis release a significant amount of hazardous substances including PAHs (Ross et al. 2002). Pyrogenic PAHs are typically formed at elevated temperature (350–1,200°C), although they can also be emitted from a low temperature (100–150°C) combustion process. Pyrogenic sources can be natural or anthropogenic. Many natural events, for instance, forest burning, bushfire, and volcanic eruption, release PAHs into the environment (Menzie et al. 1992; Zhang and Tao 2009). Anthropogenic emission sources can be divided further into four major subclasses based on the sources: domestic, industrial, automotive exhaust, and agricultural. Domestic emission results from cooking and heating activities. The burning of coal, oil, gas, garbage, wood, and other organic substances emit PAHs. Cigarette smoke, fireplace, and backyard barbecues also contribute to the emission.

Pyrogenic process is defined as high-temperature low-oxygen combustion process, domestic open burning of coal, peat, wood, straw, cow-dung-cake, rice husk briquettes, and garbage at a temperature as low as 150–200°C contributing to the emission of PAHs (Tsibart et al. 2014). Since the industrial revolution, the major portion of PAHs in the environment, especially in the ambient air, has been added directly or indirectly from industrial activities. Destructive distillation of coal to coke and coal tar, thermal cracking of petroleum residue, burning of fuels, metallurgical process such as aluminum smelting, rubber tire industry, cement manufacturing

industry, waste incineration, bitumen and asphalt production, wood preservation, commercial heating plant, as well as manufactured gas plant sites (MGP) are some of the conventional sources of pyrogenic PAHs. Automotive emission sources include exhaust gases from automobile vehicles, railway, ship, aircraft, jet engine, and other motor vehicles. Some agricultural practices, for example, intentional burning of bushland and forest, straw and stubble, and moorland heather, also contribute to the buildup of PAHs concentration in the environment (Stogiannidis and Laane 2015; Abdel-Shafy and Mansour 2016). Pyrogenic emission contains both LMW and HMW PAHs. Due to their higher vapor pressure, LMW constituents are released in gaseous form, whereas HMW PAHs are abundant in particle-sorbed form. Generally, HMW PAHs share the significant part of emitted PAHs from a pyrogenic origin (Ou et al. 2004; Page et al. 2006; Boll et al. 2008). Pyrene, fluoranthene, BaP, chrysene, and, to a lesser extent, phenanthrene are found commonly in pyrogenic emission. The predominance of these parent PAHs over their alkylated homologues is used as an indication of pyrogenic origin (Blumer and Youngblood 1975; Laflamme and Hites 1978; Sporstol et al. 1983; Wang et al. 2001).

3.2 Petrogenic Sources

Petrogenic PAHs are constituents of petroleum products including crude oil, engine oils, lubricant, and their derivatives. Petroleum fuel has become the inevitable energy source since the dawn of the industrial revolution in the late eighteenth century. Dependency on fossil petroleum fuels leads to an extensive exploration and transportation of petrochemicals. Crude oil and refined petroleum fuels are rich in parental PAHs, alkyl-PAHs, azaarenes, and thiophenes (Grimmer et al. 1983). Petrogenic PAHs enter the environment through maturation, drilling, transportation, storage, use of crude oil, and related petrochemicals. Petrogenic sources can be natural or anthropogenic. Petroleum seeps from natural crude oil reservoir and erosion of sedimentary rock release PAHs into the environment. The release of petrogenic PAHs from anthropogenic activities is a significant route of PAHs contamination. Fuel-based industrial dependency has boosted economic growth; however, indiscriminate use of fossil fuels also engenders severe environmental pollution as a result of accidental as well as the intentional release of crude oil and refined products. Some important sources of petrogenic PAHs of anthropogenic origin are oceanic and freshwater oil spills, underground or aboveground storage tank leak, oil refinery waste, and leakage of crude and refined oil during transportation (Stogiannidis and Laane 2015). Since the last few decades, marine oil spills have become a recurring disaster. Amoco Cadiz (1978), Ixtoc I (1979), Atlantic Empress (1979), Exxon Valdez (1989), Kuwaiti Oil Lakes (1991), Kuwaiti Oil Fires (1991), Gulf War (1991), and Deepwater Horizon (2010) oil spills released massive amounts of crude and refined fuel rich in PAHs in aquatic environment (Hayakawa 2018).

Although the PAHs constituents of petrogenic sources vary greatly depending on the origins, LMW PAHs are the dominant representatives. Furthermore, most of the petrogenic release contains 16 US EPA priority pollutants and alkylated forms of 5 parental PAHs (alkylated naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene). The parental compounds and "alkylated five" are used often as signature molecules for indicating petrogenic PAHs contamination of sediments (Laflamme and Hites 1978; Wang et al. 2001; Boll et al. 2008; Stogiannidis and Laane 2015). Coal tar and creosote are other important sources of PAHs. Creosote is a mixture of several hundreds of compounds; PAHs may constitute 90% of creosote. It is used widely as a wood preservative and waterproofing agent. Creosote enters soil and water mainly through wood preservation industry wastewater. Seeping and leakage of creosotes from treated timber may add PAHs in soil. A minor fraction (1–2%) of creosote is released in air through volatilization (Nestler 1974).

3.3 Diagenetic and Biogenic Sources

Not as pronounced and pernicious as pyrogenic and petrogenic sources, the biogenic and diagenetic process contribute to the environmental PAHs load. Crude oil, coal, and gases are formed from sedimentary algae, diatoms, phytoplankton, and bacteria through a process called diagenesis. Biogenic PAHs are derived from biosynthesis of the compounds in plant, phytoplankton, and microorganisms. Some endophytic fungi produce naphthalene (Daisy et al. 2002; Ezra et al. 2004). Naphthalene is also a major chemical component of *Magnolia* flower (Azuma et al. 1996). A microalga, *Chlorella vulgaris*, was found to synthesize several PAHs, including BaP, while growing in acetate-containing medium (Borneff et al. 1968).

In addition to the sources mentioned above, unburnt hard coal (bituminous coal) has recently been reported as a PAHs source (Achten and Hofmann 2009). Rochman et al. (2013) reported the sorption of several unsubstituted PAHs and their nitro- and methyl-substitutes on virgin polystyrene and polystyrene debris in marine environment suggesting that the polymers are a potential secondary source. Petrogenic sources are generally accidental and acute. They do not contribute to continuous contamination. In contrast, pyrogenic sources, especially incomplete combustion processes, are considered as the prominent and chronic sources of PAHs entering the environment (Duran and Cravo-Laureau 2016). Irrespective of sources, most of the released PAHs ultimately find their way to surface water, topsoil, the bottom surface of lakes, estuaries or rivers, and sediments either via airshed (dry and wet deposition) or watershed (e.g., urban runoff, rainfall, snow/ice fall, etc.).

4 Fate of PAHs in Aerobic Environments

The distribution, partition, transport, and fate of PAHs in the environment are intimately dependent on their sources, physicochemical properties, some environmental parameters, and biodegradation mechanisms. As their origin can be diverse, assigning a single source to a pool of PAHs in each environment is difficult (Blumer 1976). Moreover, post-emission alteration of PAHs gives rise to substitutes. As a result, the atmosphere contains a variable concentration of unsubstituted and substituted PAHs. A simplified overview of the possible fates of PAHs in the environment is presented in Fig. 3.

The fate of PAHs in air depends largely on temperature, humidity, precipitation, sunlight, and presence of atmospheric gases and acids. Temperature and humidity are the two main factors that govern the ratio of gas-to-particulate PAHs in the air (Maliszewska-Kordybach 1999). PAHs transformation in the atmosphere occurs

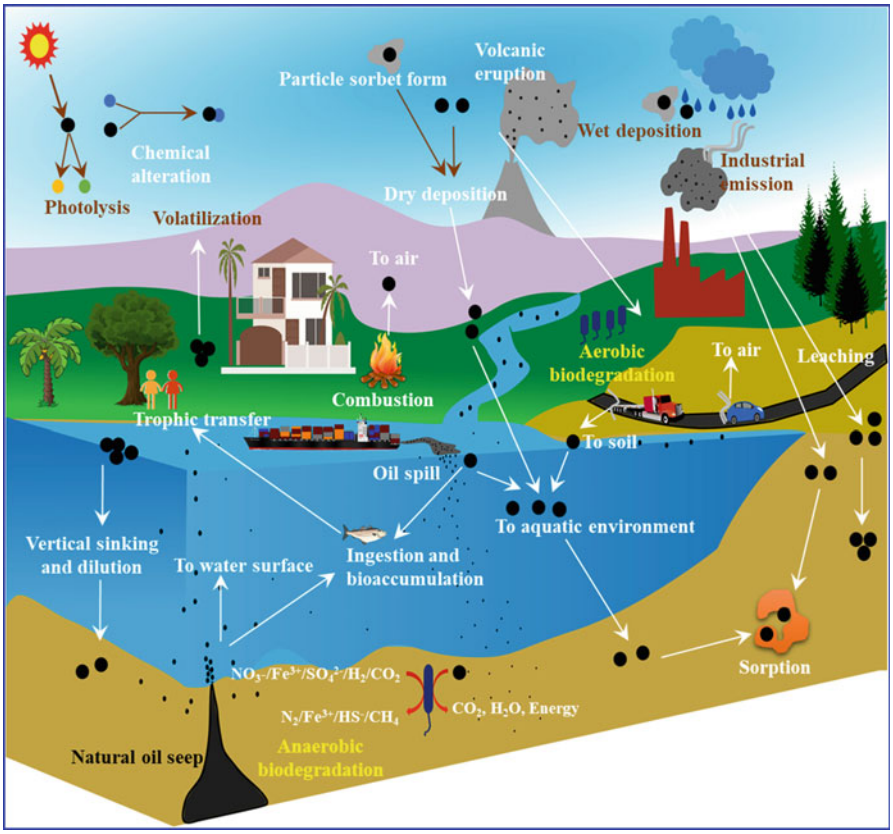

Fig. 3 The possible fates of PAHs (in black dots) in the environment. Among the several possibilities, the most common ways of origin, transfer, and removal are illustrated

mainly by chemical reactions and physical deposition. Reaction with ozone, nitrate and hydroxyl radicals, and acids derived from NOx and SOx and photolysis are accounted for the loss of gas-phase PAHs (Valerio et al. 1984). Alkyl-substituted PAHs are common constituents of crude and refined oil. Petroleum products are formed in sediment over a long period under pressure and temperature (150–200°C). Such comparatively mild temperature range favors the formation of alkylated derivatives so vigorously that the relative amounts of alkyl-PAHs may far exceed their parent compounds. Alkyl-PAHs have been found as the major pollutant in environments contaminated with crude and petroleum oil. Several methylated PAHs have been identified in urban air, street dust, and sediment. Alkyl-PAH also enter the environment through petrogenic, pyrogenic, and industrial sources (Miki et al. 2014; Tuyen et al. 2014; Wei et al. 2015). Nitro-PAHs are formed by nitration of parent PAHs during incomplete combustion or atmospheric gas-phase reaction. They enter the environment from automobile exhaust, waste incinerator, and domestic wood burning (Lima et al. 2005; Karavalakis et al. 2010; Shen et al. 2012; Bandowe and Meusel 2017). Oxygenated PAHs (oxy-PAHs) have one or more oxygen atom(s) attached to the aromatic structure of parent PAHs. Incomplete combustion is one of the major sources of oxy-PAHs. Parent PAHs may also transform to oxy-PAHs through light-induced reaction and chemical oxidation with singlet oxygen, peroxides, peroxyl radical, and hydroxyl radicals (Lundstedt et al. 2007). Particle-phase PAHs are also subjected to similar reactions. Most of the atmospheric PAHs deposit near their sources; however, PAHs with higher vapor pressure are transported to far away from their sources and are distributed worldwide. PAHs were detected in remote areas like Antarctic snow (Kukučka et al. 2010) and the Antarctic and Southern sea atmosphere (Cabrerizo et al. 2014). Physical removal of PAHs from the atmosphere generally occurs through dry deposition and wet deposition. In a dry deposition, PAHs are adsorbed on particulates and settle down slowly due to gravitational pull. The process depends on the size/mass of the particle and some environmental factors such as seasonal temperature, wind speed, turbulence, etc. Due to comparatively high aqueous solubility, atmospheric vapor-phase LMW PAHs may dissolve in cloud and raindrop that upon condensation of clouds settle down on Earth's surface during precipitation. Particle-sorbed PAHs also settle on the surface through wet deposition. Transport of gas-phase PAHs from temperate or tropical warm regions of Earth to high-latitude cold regions is governed mainly by atmospheric temperature and vapor pressure of PAHs. The phenomenon can be explained by the global distillation effect theory. It predicts that atmospheric gas PAHs are transported to colder regions and condense. The deposited compounds may undergo several volatilization-transport-deposition cycles. This multiple hopping from low latitude to high latitude is known as the grasshopper effect. Consequently, PAHs would reach to the polar regions where low ambient temperature prevents their further transport; the effect is termed as "cold trap" or "cold finger" (Wania and Mackay 1993, 1995, 1996; Fernández and Grimalt 2003). Although not all atmospheric PAHs are removed, a significant portion finds their way to soil, water surface, and vegetation (Maliszewska-Kordybach 1999).

Soil can be contaminated with PAHs through different ways. Notable routes include dry and wet atmospheric deposition, automobile exhaust, sewage sludge, industrial effluent, seeping and leakage from coal tar creosote impregnated timbers, unburnt bituminous coal, roadway asphalts, accidental release of crude or refined oil during transportation and handling. PAHs in the soil can have different fates depending on the physical and chemical properties of PAHs, soil texture, soil organic matter (SOM) content, environmental conditions, and associated removal mechanisms. Sorption, sequestration, evaporation, photolysis, leaching, as well as biodegradation are the primary routes of PAHs processing in the soil. Sorption and sequestration processes play a significant role in PAHs accumulation in soil. Due to the strong sorption capacity of SOM and minerals, PAHs tend to be fixed with these substances (Means et al. 1980). The content, composition, and structure of SOM influence the sorption process. Also, soil particle size, the presence of clay minerals, and soil pH determine the extent of PAHs sorption to soil. Rhizosphere soil facilitates sorption of PAHs as root exudates increase soil structure (Wilcke 2000; Okere and Semple 2012). Evaporation or volatilization largely depends on daily and seasonal temperature. At elevated temperature, PAHs, mostly from topsoil, may evaporate quickly. Air current disperses the evaporated PAHs to a lower temperature region where they settle down through wet deposition (Sims and Overcash 1983; Wild and Jones 1995). Unlike atmospheric PAHs, very little soil PAHs are transformed through light energy. If any photo-destruction occurs, it remains confined to few millimeters of topsoil only. Therefore, photooxidation is not considered as a significant way of PAHs processing in the soil (Sims and Overcash 1983). Due to lower aqueous solubility and decreased mobility, leaching of unsubstituted PAHs is limited in the soil. However, semipolar derivatives (nitro-, oxy-, hydroxy-PAHs) show increased mobility in soil and hence dissipate to the soil column through micropores (Sims and Overcash 1983). Lipophilic nature of both PAHs and plant cuticle facilitate the accumulation of significant amounts of particle-bound PAHs in leaves, trunk, needles, and bark. Accumulated PAHs can enter the soil through plant litters during or at the end of vegetation period, precipitation, and near-stem runoff. Plant root system can uptake PAHs, and gas-phase PAHs may be accumulated by plant stroma and subsequently transported through vascular system or by diffusion; the cycle is completed at the end of vegetation (Wilcke 2000).

The marine environments including estuaries, coastal areas, ocean surface, and deep-sea shelter are diverse ecosystems. The fate of PAHs in the marine environment is determined mainly by the mode of PAHs entrance. Also, physical and chemical properties of PAHs, the presence of co-contaminants, sediment composition, environmental conditions, and hydrologic dynamics also influence PAHs fate in the marine environment (Latimer and Zheng 2003). Pyrogenic PAHs from combustion sources enter through urban runoff and atmospheric deposition. The accidental oil spill has become a significant means of PAHs entrance into the marine environment. Crude oil release from natural oil seep is the other notable source of PAHs in the environment. In the marine environment, petrogenic PAHs are more bioavailable because of the abundance of LMW PAHs, while pyrogenic PAHs are more recalcitrant as they remain sorbed to organic particulate matters. PAHs in the

marine environment are transformed in abiotic processes such as volatilization, photooxidation, and chemical alteration. A significant portion of PAHs sinks vertically to marine sediment where microbial degradation becomes the primary fate of the pollutant. However, due to the hydrophobic nature of PAHs, they tend to become sorbed to sediment organic matters and mineral particles and thus become less bioavailable, hence persist in the marine environment (Acosta-González and Marqués 2016; Duran and Cravo-Laureau 2016).

Biodegradation is the principal mechanism for removing PAHs from the soil. The uptake and degradation of PAHs by microorganisms depend largely on soil temperature and other physicochemical properties of PAHs and the nature of the organisms. Bacteria, algae, and fungi can degrade many PAHs and their derivatives. Plants can also extract, sequester, and detoxify PAHs from the environment. In general, bacteria utilize PAHs as a carbon and energy source. Fungal degradation process, in contrast, leads to detoxification rather than mineralization (Cerniglia 1992; Samanta et al. 2002; Haritash and Kaushik 2009; Cerniglia and Sutherland 2010).

A growing body of literature deals with the bacterial degradation of PAHs from soil, water, and sediment under aerobic, microaerobic, and anaerobic conditions. Aerobic degradation of PAHs has been outlined in some excellent reviews (Cerniglia 1992; Juhasz and Naidu 2000; Kanaly and Harayama 2000; Peng et al. 2008; Haritash and Kaushik 2009). Aerobic bacterial degradation of PAHs, especially 2–5 ring PAHs, has been investigated well. Members of the genera, *Pseudomonas*, *Sphingomonas*, *Mycobacteria*, *Burkholderia*, *Rhodococcus*, *Flavobacterium*, *Acinetobacter*, and *Klebsiella*, have been frequently isolated from contaminated sites. Aerobic degradation of PAHs by bacteria involves the introduction of both atoms of oxygen to the aromatic structure producing *cis*-dihydrodiols. The enzyme dioxygenase is a multicomponent protein consisting of ferredoxin, ferredoxin reductase, and an iron-sulfur protein (Habe and Omori 2003). The resulting *cis*-dihydrodiols are then rearomatized to dihydroxylated intermediates by the action of dehydrogenases. Ring cleavage of the intermediates produce TCA cycle intermediates and finally mineralized to CO_2 and H_2O with the production of energy. In addition, identification of *trans*-dihydrodiol metabolites during PAHs degradation by certain strains of *Mycobacterium* and *Streptomyces* suggests cytochrome P450 oxygenases-mediated transformation also accounts for PAHs metabolism in bacteria (Sutherland et al. 1990; Tongpim and Pickard 1999).

Many fungi have been reported to degrade PAHs. In most of the cases, fungal degradation of PAHs is cometabolic. However, some fungi can utilize PAHs as the sole sources of carbon. For example, *Fusarium solani* was able to germinate on and mineralize BaP (Rafin et al. 2000). PAHs-degrading fungi generally belong to two major groups: (a) ligninolytic fungi that produce extracellular enzymes to degrade wood derived lignin and (b) non-ligninolytic fungi that do not possess lignin-degrading enzyme system. Lignin is a class of complex HMW compounds found in the vascular tissue of plants and some algae. Lignin bears structural resemblance to PAHs. Due to structural irregularity, lignin-decomposing enzymes show low substrate specificities; these enzymes can catalyze the transformation of several organic pollutants including PAHs. Among the wood-decaying and

lignin-decomposing fungi, "white-rot fungi" has been studied extensively. Notable members of this group are *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Pleurotus ostreatus*. The ligninolytic enzyme system involved in PAHs degradation comprises one or more of two glycosylated heme-containing peroxidases, lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), and a copper-containing phenoloxidase, laccase. LiP oxidizes PAHs in the presence of H_2O_2, MnP oxidizes PAHs using Mn-dependent peroxidation of unsaturated lipids, whereas laccase oxidizes PAHs in the presence of phenol, aniline, 4-hydroxybenzoic acid, methionine, cysteine, or reduced glutathione as mediator. PAHs biodegradation by white-rot fungi initiates with the generation of hydroxyl free radical by the donation of one electron, which oxidizes the PAHs ring. The reaction products include PAH quinone and acids, which may be further metabolized to nontoxic intermediates or end products via ring fission (Cerniglia and Sutherland 2010).

Many non-ligninolytic hyphomycetes, zygomycetes, and ascomycetes can metabolize PAHs. Several species of *Aspergillus*, *Penicillium*, *Fusarium*, and *Cunninghamella* have been reported to transform and sometime mineralize PAHs. Many of these fungi utilize intracellular cytochrome 450 monooxygenases system that initiates PAHs metabolism through ring epoxidation reaction producing epoxide and water. The unstable epoxide is hydrated by an epoxide hydrolase to form *trans*-dihydrodiol or rearranged to phenol derivatives by nonenzymatic action. The reaction products, PAH *trans*-hydrodiol and phenols, are then methylated or form conjugates with sulfate, xylose, glucuronic acid, or glucose. Ligninolytic fungi may also involve in PAHs metabolism through the production of intracellular cytochrome P450 and epoxide hydrolase (Cerniglia and Sutherland 2010). Unfortunately, some fungal metabolites are more toxic than the substrate PAHs. Vázquez-Duhalt et al. (2001) reported the conversion of PAHs to mono-, di-, and tri-chlorinated compounds by the chloroperoxidase enzyme of *Caldariomyces fumago* in the presence of H_2O_2 and chloride ion. Some of these chlorinated compounds were more mutagenic than their parent PAHs.

PAHs are toxic to many aquatic animals and plants (Landrum et al. 1986; Yu 2002). Nevertheless, algal biotransformation of PAHs along with bacterial and fungal degradation is an important determinant of the fate of PAHs in the aquatic environment. Both fresh and marine water algae can degrade PAHs. A cyanobacterial strain, *Agmenellum quadruplicatum* PR-6, and a microalga, *Oscillatoria* sp. JCM, can oxidize naphthalene to 1-naphthol (Cerniglia et al. 1979, 1980). The green algae, *Selenastrum capricornutum*, *Scenedesmus acutus*, and *Ankistrodesmus braunii*, metabolize BaP through dioxygenase pathway and produce dihydrodiols and quinones. The degradation extent and metabolites were found to depend on light intensities, algal species, and dose (Schoeny et al. 1988; Warshawsky et al. 1995). Two diatoms, *Skeletonema costatum* and *Nitzschia* sp., isolated from mangrove aquatic ecosystem, were reported to accumulate and degrade phenanthrene and fluoranthene (Hong et al. 2008). Interesting enough, dead cells of algae retain PAHs removal capability. Lei et al. (2002) reported no significant differences in the removal of pyrene by live and dead cells of *Chlamydomonas* sp., *Chlorella miniata*, *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*,

S. capricornutum, and *Synechocystis* sp. This study suggested both biosorption (by dead cells) and bioaccumulation (inside live cells) as PAHs removal mechanisms by the microalgal cultures. Similarly, dead cells of *S. capricornutum* exhibited removal of several HMW PAHs including benz(a)anthracene, BaP, dibenzo(a,h) anthracene, indeno(1,2,3-cd)pyrene, and benzo(g,h,i)perylene (Luo et al. 2014). The same research team established that photocatalytic transformation of BaP is catalyzed by chlorophyll of dead algal cells through the formation of a high level of reactive singlet oxygen species (Luo et al. 2015). Soil microalgae have also been reported to degrade PAHs. A soil microalga, *Chlorella* sp. MM3, has recently been reported to degrade pyrene from both liquid media and soil slurry (Subashchandrabose et al. 2017). Algal transformation of PAHs is species-specific (Kirso and Irha 1998), and bacterial-algal consortia have been considered better suited than monoculture in the removal of PAHs from the environment (Warshawsky et al. 2007).

In addition to microbial degradation, several plants including grasses have been reported to play a role in the removal of PAHs from the environment. As with microbial bioremediation, phytoremediation of PAHs has been gaining recognition as an efficient pollutant-remediation technique (Sivaram et al. 2018). Plant-mediated transformation of PAHs involves the uptake of the pollutants from contaminated soil to the plant system through the root, translocation within the plant tissues, enzymatic breakdown or modification, conjugate formation, sequestration of conjugates within plant compartment, and further processing of the conjugates (Arthur et al. 2005). Moreover, plants facilitate immobilization of PAHs in soil and promote microbial degradation. Plant-microbe association, as in rhizosphere and mycorrhiza, is another means in determining the fate of PAHs in the environment (Ma et al. 2010).

As such, very little is known about the fate of PAHs in the anaerobic environment. Fresh and marine water sediments, sewage, subsurface aquifer sediment, and groundwater contamination with PAHs occur from anthropogenic activities such as shipping, boating, fishing, oil spill, leakage of coal tar, creosote and petroleum fuel from the surface storage tank, and gas production. The absence of molecular oxygen and sorption of PAHs to sediment organic matter are probably the most critical factors that govern persistence of PAHs in such marine environment.

5 Onset of Anoxia and Nature of Anoxic Environment

Unavailability of gaseous and dissolved oxygen in an environment renders it anoxic. The atmosphere, as we know it now, consists of the essential gaseous mixture for supporting life. The strong oxidizing gas, oxygen, is an absolute requirement for aerobic respiration. It acts as the terminal electron acceptor (TEA) in the aerobic cellular respiration process and participates in many biological reactions as a co-substrate. During the formation of Earth, it experienced extended anoxia until molecular oxygen began accumulating in the atmosphere when ancient microbial life forms breathed out the gas. After millions of years of accumulation, we are now

breathing in an atmosphere consisting of ~21% oxygen (Margulis and Sagan 1997; Planavsky et al. 2014).

In well-structured and drained soil, gaseous oxygen penetrates through the cracks and pores. As a result, the topsoil layer becomes sufficiently oxygenated and supports aerobic microbial metabolism and root respiration (Drew 1990). Hydrosphere can be saturated with atmospheric oxygen to the extent that the maximum dissolved oxygen (DO) level can reach as much as 9–10 mg L^{-1} (McNeely et al. 1979). Often oxygen level becomes limited below few millimeters/centimeters along soil/sediment profile. Wet or waterlogged soil, sediment overlaid by stagnant or constant-depth water column, and subsurface groundwater aquifers generally contain very limited DO that, in many cases, reaches a zero value. Moreover, specific habitats like marine sediment remain in permanent anoxia (Kaiho 1994). Contrary to the complete aerobic and anaerobic environment, DO level in a hypoxic environment ranges between 0 and 4.5 mg L^{-1} (Wu 2002). Many habitats such as wetlands and swamps exhibit hypoxic condition. The DO level of an environment is highly influenced by temperature, salinity, and microbial activity (Brune et al. 2000). Sediment top layer receives considerable input of organic biomass from terrestrial and aquatic algae, plants, and animals. During the decomposition of organic compounds, aerobic microorganisms use available oxygen. As a result, DO level decreases with time. Once the oxygen demand of a habitat exceeds the oxygen dissolution rate, anoxia begins to develop. Moreover, continuous input of a large amount of natural and synthetic organic compounds in water, soil, or sediment environment exacerbates the DO level and creates an oxygen-depleted anaerobic condition (Burdige 2007).

A redox gradient along its depth characterizes an anoxic environment (Fig. 4). Extensive use of oxygen during microbial decomposition of organic matters fosters the formation of a redox gradient that is characterized by rapid decrease in DO level and redox potential across the gradient and the variable availability of alternate electron acceptors for microbial respiration (Fig. 4) (Brune et al. 2000; Li et al. 2009). Depending on the characteristics of the site, nitrate, manganese, iron, and sulfate become the dominant electron acceptors. The gradient starts at a transition zone where nitrate, manganese(IV), and iron(III) are used preferentially as a TEA. Down to the transition zone, sulfur reduction process turns out to be the prominent anaerobic respiration regime. The methanogenic zone may be developed further below a sulfidic regime where methane production often occurs through interspecies syntrophic metabolism (Cappenberg 1974; Acosta-González and Marqués 2016).

In the anoxic environment, anaerobes are the key players in geochemical cycling. Cellular respiration in oxygen-depleted conditions is challenging because oxygen is no longer available or "died out." The Gibbs free energy ($\Delta G^{\circ\prime}$) change in the oxidation of NADH ($E^{0\prime} = -320$ mV) coupled with oxygen reduction to water ($E^{0\prime} = +818$ mV) is -220 KJ mol^{-1}. When the same reaction coupled with NO_3^- reduction to NO_2^- ($E_0' = +433$ mV) and CO_2 reduction to CH_4 ($E_0' = -244$ mV), the free energy changes decrease by -56.2 KJ mol^{-1} and -157.2 KJ mol^{-1} for the respective processes (Thauer et al. 1977). Microorganisms being the first forms of life on Earth show adaptation to a range of oxygen environments. On the contrary,

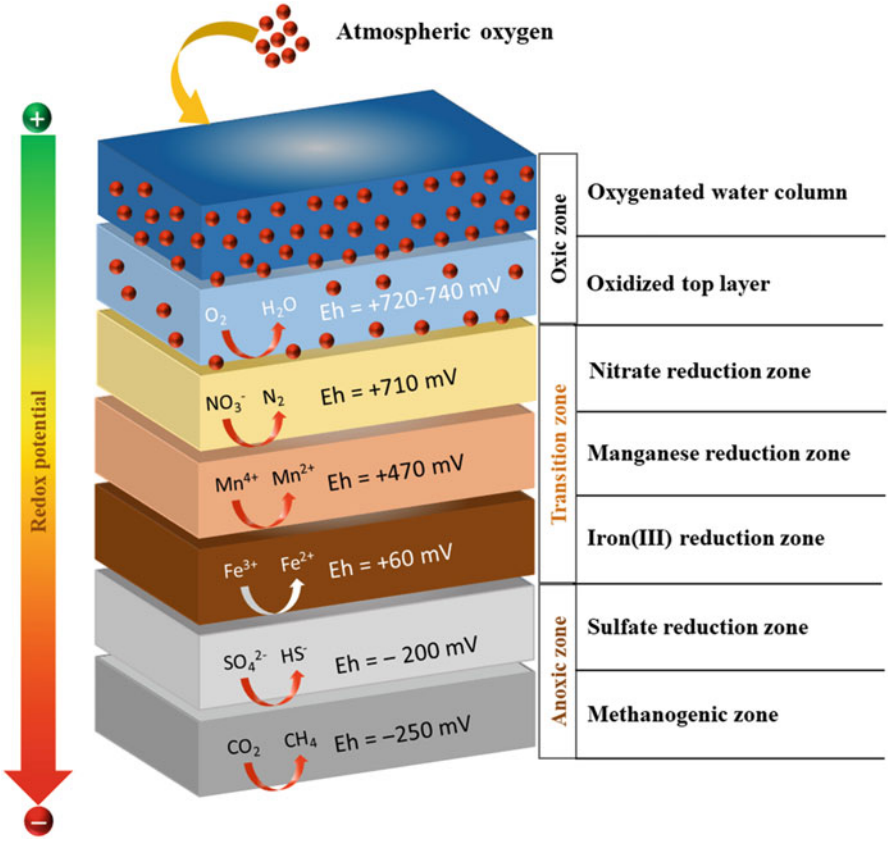

Fig. 4 Schematic of a typical sediment column characterized by decreasing redox gradient below the oxidized zone. In the transition zone, microbial respiration processes use NO_3^-, NO_2^-, Mn^{4+}, Fe^{3+}, and SO_4^{2-} as terminal electron acceptors. With increasing depth, redox potential drops dramatically, and sulfur reduction becomes predominant in the high negative redox potential zone. Further down to this sulfidic zone, methanogenic activity by strict anaerobic bacteria and archaea may be present

some of them are fitted out at surviving in the total absence of oxygen. It comes with a surprise to think that the first life form, no matter whether it emerged in an ancient "pond of soup" or in a hydrothermal vent (Nisbet and Sleep 2001; Weiss et al. 2016), might be capable of thriving under anoxic conditions. Still, we know very little about the physiology and metabolism of anaerobic microorganisms. Facultative anaerobes are the significant occupants in the transition zones, while strict anaerobes govern biogeochemical cycling at sulfate-reducing and methanogenic zones (Lovley 2001). Down to the gradient, the reduction potential drops abruptly, and the net energy yield during oxidation, per molecule, of organic matter decreases strongly. Anaerobic lifestyle, therefore, should be parsimonious enough to allow efficient survival, maintenance, and cell growth with a limited amount of available energy. Many

anaerobic metabolisms even run close to the thermodynamic limits (Fuchs et al. 2011). Although several growth-limiting constraints exist, anaerobes contribute crucially to the global biogeochemical cycle.

6 Persistence of PAHs Under Anaerobic Conditions

In an anaerobic environment, PAHs are susceptible to microbial enzymatic degradation if they are bioavailable, enough appropriate inorganic electron acceptors are present, and the native microflora possess genetic setup for encoding necessary degradative enzymes. The absence of one or more of the prerequisites may affect PAHs degradation process that consequently would lead to recalcitrance and accumulation of the pollutants in the environment. Additionally, PAHs often occur with other mixed contaminants in real impacted sites. The presence of other toxic substances such as heavy metals, cyanides, and organic compounds can impede PAHs degradation (Kuppusamy et al. 2017).

Under anaerobic condition, PAHs may resist microbial degradation. Sharak Genthner et al. (1997) reported the persistence of many PAHs tested in their study under various redox conditions. Only little degradation of naphthalene, 1-methylnaphthalene (MN), and 2-MN occurred under methanogenic conditions. In a methanogenic sediment column, benzene as well as naphthalene remained persistent for 20–40 days even after the addition of simpler co-substrates such as acetate, benzoate, lactate, and phenol (Langenhoff et al. 1996). Bauer and Capone (1985) observed that although anthracene and phenanthrene were degraded relatively well under aerobic conditions, they remained persistent under anaerobic conditions. In another study, HMW PAHs in contaminated arctic soils remained refractory to biodegradation under nitrate-reducing conditions at both low (7°C) and moderate (20°C) temperature. Under aerobic conditions, however, the HMW PAHs were degraded well (Eriksson et al. 2003). Recently, Folwell et al. (2016) reported persistence of pyrene and naphthenic acids in oil-sand-water from tailing pond. Failure to establish PAHs-degrading enrichment culture in laboratory microcosm study may be attributed to the absence of appropriate electron acceptor, reducing conditions and essential nutrients in the culture media. In addition, very low or total absence of requisite microbial population and their slow adaptation to the contaminants may also lead to the failure in developing enrichments. Inhibition of degradation by co-occurring contaminants may also contribute to the apparent persistence of PAHs in the environment or a laboratory microcosm.

7 Feasibility of Anaerobic Biodegradation of PAHs

The stabilizing resonance energy of the aromatic compounds is the major hindrance to microbial degradation. Moreover, unavailability of oxygen in an anaerobic environment presents another critical challenge to microorganisms that require to use

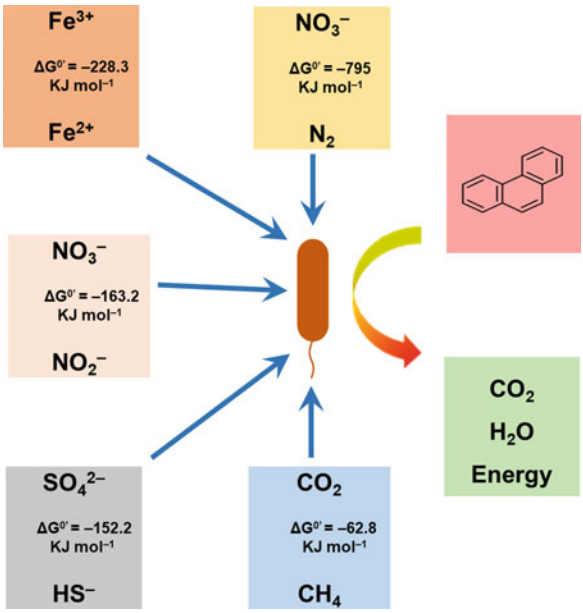

Fig. 5 Conceptual representation of anaerobic degradation of PAHs under various reducing conditions. The standard Gibbs free energy changes ($\Delta G^{\circ\prime}$) for the redox couples under standard conditions and pH 7.0 in reduction processes of electron acceptor are obtained from Thauer et al. (1977)

aromatic compounds as growth substrates (Fuchs et al. 2011). In the absence of oxygen, where it is no longer available to accept electrons during respiratory electron transport, anaerobes use several inorganic ions or compounds as TEA (Fig. 5). Nitrate is one of the first components in anaerobic nitrate respiration process; facultative nitrate reducers can harness energy by reducing nitrate to different nitrogen oxides and molecular dinitrogen. From a thermodynamic point of view, standard Gibbs free energy change in nitrate reduction process is close to that in aerobic respiration process (-220 KJ mol^{-1} vs -163.2 KJ mol^{-1}) (Thauer et al. 1977). Thus, nitrate reduction is a widespread process often associated with degradation of POPs. Nitrate-reducing microorganisms mainly belong to the facultative anaerobic group of bacteria. If available, oxides of manganese and iron(III) can also act as TEA in anaerobic metabolism. Contaminants often move down to the gradient to the utterly anoxic zone. SRB often degrade organic compounds in strictly anaerobic conditions. Fortunately, sulfate is abundant in many anaerobic systems such as marine sediment. SRB are considered the oldest life forms that can be dated back to 3.5 billion years. Soon after the formation of Earth, SRB have been contributing to the biogeochemical cycling (Barton and Fauque 2009).

The primary challenge in organic pollutant degradation through sulfur reduction process is low changes in Gibbs free energy. Despite the limitation, many SRB are known to degrade many POPs including PAHs. For a long time, anaerobic degradation of hydrocarbon compounds under methanogenic conditions was considered as thermodynamically improbable. However, the evidence is accumulating in support of the methanogenic degradation of crude oil in the deep subsurface environment (Aitken et al. 2004). Dolfing et al. (2009) calculated free energy change during

the oxidation of PAHs under methanogenic conditions. The $\Delta G^{\circ\prime}$ values for methanogenic naphthalene, phenanthrene, anthracene, pyrene, and chrysene degradation ranged from −208.8 to −331.4 kJ mol^{-1}, and the energy yield per mole CH_4 generation was in the range of −27.1 to −34.8 kJ mol^{-1}. Although the reaction is exergonic, sharing of energy among the associated interdependent microbial members of an anaerobic syntrophic metabolism makes the process challenging. The authors predicted that oxidation to H_2/CO_2 or conversion to acetate is energetically more favorable for PAHs degradation under methanogenic conditions. The calculation of free energy changes under standard conditions (25°C, atmospheric pressure) during anaerobic oxidation of four model PAHs indicates that the anaerobic oxidation processes under nitrate-reducing, sulfate-reducing, and methanogenic conditions are exergonic (Table 2). The Gibbs free energy change and the ATP produced per mole of substrate oxidation for any of the PAHs are the highest under denitrifying process and lowest under methanogenic conditions. With increasing molecular weight, energy yield also increases. However, the thermodynamic calculation is based on differences between the formation energy of reactants and products. Therefore, calculations of free energy changes under standard conditions

Table 2 Reaction stoichiometry, free energy change at standard conditions (at 1 M concentration of solute and 25°C) during anaerobic oxidation of naphthalene, phenanthrene, pyrene, and benzo(a) pyrene (BaP) under nitrate-reducing, sulfate-reducing, and methanogenic conditions

TEA	Stoichiometric equation	$\Delta G^{\circ\prime}$ (KJ)[a]
Naphthalene		
NO_3^-/N_2	$C_{10}H_8 + 1.2H_2O + 9.6NO_3^- \rightarrow 10HCO_3^- + 0.4H^+ + 4.8N_2$	−4,783.35
SO_4^{2-}/HS^-	$C_{10}H_8 + 6SO_4^{2-} + 6H_2O \rightarrow 10HCO_3^- + 6HS^- + 4H^+$	−461.81
CO_2/CH_4	$C_{10}H_8 + 18H_2O + 6CO_2 \rightarrow 10HCO_3^- + 6CH_4 + 10H^+$	−188.72
Phenanthrene		
NO_3^-/N_2	$C_{14}H_{10} + 2.4H_2O + 13.2NO_3^- \rightarrow 14HCO_3^- + 0.8H^+ + 6.6N_2$	−6,591.96
SO_4^{2-}/HS^-	$C_{14}H_{10} + 8.25SO_4^{2-} + 9H_2O \rightarrow 14HCO_3^- + 8.25HS^- + 5.75H^+$	−649.84
CO_2/CH_4	$C_{14}H_{10} + 25.5H_2O + 8.25CO_2 \rightarrow 14HCO_3^- + 8.25CH_4 + 14H^+$	−274.35
Pyrene		
NO_3^-/N_2	$C_{16}H_{10} + 3.6H_2O + 14.8NO_3^- \rightarrow 16HCO_3^- + 1.2H^+ + 7.4N_2$	−7,626.95
SO_4^{2-}/HS^-	$C_{16}H_{10} + 9.25SO_4^{2-} + 11H_2O \rightarrow 16HCO_3^- + 9.25HS^- + 6.75H^+$	−764.58
CO_2/CH_4	$C_{16}H_{10} + 29.5H_2O + 9.25CO_2 \rightarrow 16HCO_3^- + 9.25CH_4 + 16H^+$	−343.56
BaP		
NO_3^-/N_2	$C_{20}H_{12} + 4.8H_2O + 18.4NO_3^- \rightarrow 20HCO_3^- + 1.6H^+ + 9.2N_2$	−9,235.56
SO_4^{2-}/HS^-	$C_{20}H_{12} + 11.5SO_4^{2-} + 14H_2O \rightarrow 20HCO_3^- + 11.5HS^- + 8.5H^+$	−952.61
CO_2/CH_4	$C_{20}H_{12} + 37H_2O + 11.5CO_2 \rightarrow 20HCO_3^- + 11.5CH_4 + 20H^+$	−429.19

TEA terminal electron acceptor

[a]The standard Gibbs free energy of formation ($\Delta_f G^\circ$) for naphthalene$_{(s)}$ (252.38 KJ mol^{-1}), phenanthrene$_{(s)}$ (383.08 KJ mol^{-1}), pyrene$_{(s)}$ (491.18 KJ mol^{-1}), and BaP$_{(s)}$ (621.88 KJ mol^{-1}) is calculated according to the group contribution method as described by Joback and Reid (1987). Formation energy for other compounds, gases, and ions is obtained from Thauer et al. (1977)

are not directly applicable to environmental conditions. In a real environment, the biodegradability of PAHs depends on some factors that are not considered in a free energy change calculation. Such factors include molecular weight and conformation-related properties such as solubility, $\log K_{OW}$, affinity to organic matter, and bio-availability. Nevertheless, Table 2 indicates the feasibility of anaerobic degradation of PAHs such as naphthalene, phenanthrene, pyrene, and BaP.

8 PAHs Biodegradation Under Nitrate-Reducing Conditions

8.1 PAHs Degradation by Nitrate-Reducing Bacteria

Nitrate reduction is a crucial microbial respiration process that is often adopted by facultative anaerobes in many organic-rich and oxygen-depleted environments. The process produces enough energy that is comparable to aerobic respiration as the reduction potential of nitrate is close to oxygen. Denitrification process leads to the conversion of nitrate to dinitrogen via various oxides of nitrogen (Kuypers et al. 2018). Nitrate-reducing bacteria are versatile aromatic hydrocarbon degraders. Benzene degradation under the nitrate-reducing condition is a well-documented process (Majora et al. 1988; Nales et al. 1998; Burland and Edwards 1999; Coates et al. 2001; Folwell et al. 2016). Mihelcic and Luthy (1988a) were the first to demonstrate PAHs degradation under nitrate-reducing conditions. In this study, aqueous-phase concentration of spiked naphthalene and acenaphthene did not change during anaerobic incubation without an external electron acceptor. When nitrate was added to the culture medium, complete degradation of naphthalene and acenaphthene was observed despite a lag of about 2 weeks. Since then, PAHs degradation by several nitrate-reducing enrichments and pure cultures has been reported so far. Table 3 summarizes many of the available reports on PAHs degradation under nitrate-reducing conditions.

PAHs degradation under nitrate-reducing conditions is widespread in nature: from pristine to contaminated samples, temperate to arctic soils, freshwater to marine sediment, petrochemical to sewage sludge, etc. (Mihelcic and Luthy 1988b; Al-Bashir et al. 1990; Leduc et al. 1992; Murphy et al. 1995; MacRae and Hall 1998; McNally et al. 1998; Rockne and Strand 1998, 2001; Rockne et al. 2000; Chang et al. 2003; Eriksson et al. 2003; Ambrosoli et al. 2005; Dou et al. 2009; Lu et al. 2011; Yang et al. 2013; Liang et al. 2014; Qin et al. 2017, 2018). Most of these studies used classical ecology approach, i.e., microcosm incubation, to investigate PAHs degradation using contaminated or uncontaminated samples, non-reduced mineral salt, nitrate as the TEA and PAHs as the electron donor. Rockne and Strand (1998) adopted a fluidized bed reactor (FBR) approach for enriching PAHs-degrading nitrate-respiring bacteria (NRB). Langenhoff et al. (1996) studied naphthalene degradation in a soil percolation column. Also, PAHs degradation by pure

Table 3 Bacterial degradation of PAHs under nitrate-reducing conditions

Substrate PAH	Origin of sample	Contaminants	Initial PAH conc. (μM)	Inoculum	NO_3^- conc. (mM)	Incubation temperature (°C)	Incubation period (day)	Degradation rate (μM day^{-1})	Reference
Ace	Uncontaminated soil	NA	2.60	Soil	1.2	25	40	0.06	Mihelcic and Luthy (1988a)
Ace	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 μmol kg^{-1}	Sediment enrichment	NA	25	30	2.67 μmol kg^{-1} day^{-1}	Lu et al. (2012)
Ace	Petrochemical sludge	NA	6.48 mg kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.54 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Ace	Municipal sewage sludge	NA	6.48 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.06 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Acen	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	2.73 mg kg^{-1} day^{-1}	Lu et al. (2012)
Ant	Uncontaminated sediment	NA	0.25	*Pseudomonas putida* KBM-1	0.96	20	2.5	0.10	McNally et al. (1998)
Ant	Creosote contaminated soil	NA	0.25	*Pseudomonas fluorescens* W-2	0.96	20	1.67	0.15	McNally et al. (1998)
Ant	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	2.33 mg kg^{-1} day^{-1}	Lu et al. (2012)
Ant	Petrochemical sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.08 μmol kg^{-1} day^{-1}	Chang et al. (2003)

(continued)

Table 3 (continued)

Substrate PAH	Origin of sample	Contaminants	Initial PAH conc. (μM)	Inoculum	NO_3^- conc. (mM)	Incubation temperature (°C)	Incubation period (day)	Degradation rate (μM day^{-1})	Reference
Ant	Municipal sewage sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.1 μmol kg^{-1} day^{-1}	Chang et al. (2003)
BaP	Coking plant soil	313.86 mg/kg PAHs	99.08	*Cellulosimicrobium cellulans* CWS2	11.76	35	15	5.28	Qin et al. (2018)
BaP	Coking plant soil	313.86 mg/kg PAHs	39.63	*C. cellulans* CWS2	11.76	35	15	2.11	Qin et al. (2018)
BaP	Contaminated harbor sediment	Crude oil and PAHs	39.60	*Pseudomonas* sp. JP1	15	25	40	0.3	Liang et al. (2014)
BaP	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	1.83 μmol kg^{-1} day^{-1}	Lu et al. (2012)
Chry	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	1.77 mg kg^{-1} day^{-1}	Lu et al. (2012)
Flu	Petrochemical sludge	NA	6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.44 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Flu	Municipal sewage sludge	NA	6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.07 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Fluoran	Contaminated harbor sediment	Crude oil and PAHs	49.45	*Pseudomonas* sp. JP1	15	25	40	0.56	Liang et al. (2014)
Fluoran[a]	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	2.5 mg kg^{-1} day^{-1}	Lu et al. (2012)

Fluoran	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	2.26 mg kg^{-1} day^{-1}	Lu et al. (2012)
Nap	Creosote-contaminated harbor sediment	Creosote	6.50	Enrichment culture	3.5	20	30	2.4 mg (g of VSS $day)^{-1}$	Rockne and Strand (1998)
Nap	Uncontaminated soil	NA	54.60	Soil-water	1.2	25	45	1.2	Mihelcic and Luthy (1988a)
Nap	Gasoline-contaminated soil	PAHs and other gasoline compounds	28.90	Mixed bacterial culture	400	20	25	0.5	Dou et al. (2009)
Nap	Gasoline-contaminated soil	PAHs and other gasoline compounds	71.00	Mixed bacterial culture	400	20	25	0.78	Dou et al. (2009)
Nap	Gasoline-contaminated soil	PAHs and other gasoline compounds	100.00	Mixed bacterial culture	400	20	25	1.0	Dou et al. (2009)
Nap	Gasoline-contaminated soil	PAHs and other gasoline compounds	143.60	Mixed bacterial culture	400	20	25	1.15	Dou et al. (2009)
Nap	Gasoline-contaminated soil	PAHs and other gasoline compounds	181.00	Mixed bacterial culture	400	20	25	1.4	Dou et al. (2009)
Nap	Gasoline-contaminated soil	PAHs and other gasoline compounds	223.90	Mixed bacterial culture	400	20	25	1.76	Dou et al. (2009)

(continued)

Table 3 (continued)

Substrate PAH	Origin of sample	Contaminants	Initial PAH conc. (μM)	Inoculum	NO_3^- conc. (mM)	Incubation temperature (°C)	Incubation period (day)	Degradation rate (μM day^{-1})	Reference
Nap	Uncontaminated and weathered oil-contaminated soils	NA	390.00	Soil slurry	224.3	35	50	10.15	Al-Bashir et al. (1990)
Nap	Contaminated marine sediment	Gasoline and PAHs	78.00	Enriched bacteria culture	4.76	25	8	9.06	Lu et al. (2012)
Nap	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	2.83 mg kg^{-1} day^{-1}	Lu et al. (2012)
Nap	Contaminated soil and river sediment	Benzene, toluene and naphthalene	25 μM[b]	As is	10	20	525	70% removal at day 520[c]	Langenhoff et al. (1996)
Nap[d]	Creosote-contaminated harbor sediment	Creosote	1.6 μCi	Enrichment culture	3.5	20–25	82	17.21% recovery of radioactivity as $^{14}CO_2$	Rockne and Strand (2001)
Phe	Creosote-contaminated harbor sediment	Creosote	4.50	Enrichment culture	3.5	20	13	1.1 mg (g of VSS $day)^{-1}$	Rockne and Strand (1998)
Phe[d]	Creosote-contaminated harbor sediment	Creosote	0.4 μCi	Enrichment culture	3.5	20–25	20	96% recovery of radioactivity as $^{14}CO_2$	Rockne and Strand (2001)
Phe	Uncontaminated sediment	NA	5.61	*Pseudomonas putida* KBM-1	0.96	20	1.83	3.06	McNally et al. (1998)
Phe	Creosote-contaminated soil	NA	5.61	*Pseudomonas stutzeri* SAG-R	0.96	20	0.5	11.22	McNally et al. (1998)
Phe	Contaminated harbor sediment	Crude oil and PAHs	56.10	*Pseudomonas* sp. JP1	15	25	40	0.07	Liang et al. (2014)
Phe	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	2.43 mg kg^{-1} day^{-1}	Lu et al. (2012)

Phe	Petrochemical sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.2 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Phe	Municipal sewage sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.22 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Pyr	Uncontaminated sediment	NA	0.64	*P. putida* KBM-1	0.96	20	3	0.21	McNally et al. (1998)
Pyr	Creosote contaminated soil	NA	0.64	*P. stutzeri* SAG-R	0.96	20	1	0.64	McNally et al. (1998)
Pyr	River sediment	PAHs	49.44	*Paracoccus denitrificans*	10	28	25	1.22 (0–10 d), 1.63 (11–25 d)	Yang et al. (2013)
Pyr	River sediment	PAHs	98.89	*P. denitrificans*	10	28	25	3.58 (0–10 d), 2.05 (11–25 d)	Yang et al. (2013)
Pyr	River sediment	PAHs	197.78	*P. denitrificans*	10	28	25	8.53 (0–10 d), 0.63 (11–25 d)	Yang et al. (2013)
Pyr	River sediment	PAHs	247.22	*P. denitrificans*	10	28	25	10.8 (0–10 d), 0.08 (11–25 d)	Yang et al. (2013)
Pyr	Petrochemical sludge	NA	4.9 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.02 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Pyr	Municipal sewage sludge	NA	4.9 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.06 μmol kg^{-1} day^{-1}	Chang et al. (2003)

Ace acenaphthene, *Acen* acenaphthylene, *Ant* anthracene, *BaP* benzo(a)pyrene, *Chry* chrysene, *Flu* fluorene, *Fluoran* fluoranthene, *Nap* naphthalene, *Phe* phenanthrene, *Pyr* pyrene, *NA* not available

[a]Radiolabeled substrate

[b]Continuous flow of 25 μM naphthalene

[c]Only after the addition of 250 μM benzoate

[d]Both nitrate and sulfate as electron acceptors

bacterial cultures has also been established (McNally et al. 1998; Rockne et al. 2000; Yang et al. 2013; Qin et al. 2017, 2018).

In the natural environment, nitrate-reducing facultative anaerobes degrade both LMW and HMW PAHs. Several nitrate-reducing microcosm studies demonstrated naphthalene degradation using soil, sediment, and sludge samples. Acenaphthene, anthracene, fluorene, fluoranthene, phenanthrene, pyrene, and BaP degradation have also been reported. Al-Bashir et al. (1990) described naphthalene mineralization in both pristine and oil-contaminated soil slurry under denitrifying conditions. Naphthalene degradation was observed in a nitrate-amended sediment column only after the addition of benzoate (Langenhoff et al. 1996). McNally et al. (1998) isolated three nitrate-reducing facultative anaerobic pure bacterial cultures that could degrade acenaphthene, phenanthrene, and pyrene, both aerobically and anaerobically. A FBR enrichment culture that was developed from coal tar creosote-contaminated sediment could degrade naphthalene and phenanthrene (Rockne and Strand 1998). Subsequently, subculture was obtained through the transfer of FBR cell mass and biocarrier to PAHs amended media. The subculture showed nitrate-dependent mineralization of naphthalene and phenanthrene (Rockne and Strand 2001). Pure cultures isolated from the FBR enrichment showed higher degradation ability although at a low rate compared to the original FBR enrichments (Rockne et al. 2000). Degradation of acenaphthene, anthracene, phenanthrene, fluorene, and pyrene under nitrate-reducing conditions was also demonstrated in soil, sediment, and sludge (Chang et al. 2002, 2003; Yuan and Chang 2007). Eriksson et al. (2003) investigated anaerobic biodegradation potential of contaminated arctic soil at low temperature under nitrate-reducing conditions. Only naphthalene and 2-MN were entirely degraded by the enriched culture; fluorene and phenanthrene were also degraded to a lesser extent. HMW PAHs used in this study remained persistent at both 7 and 20°C temperature. Dou et al. (2009) demonstrated naphthalene degradation by the nitrate-reducing mixed culture at different doses. Among the 16 priority PAHs, 2- and 3-ring members have been shown to be degraded more efficiently by sediment enrichment culture under nitrate-reducing conditions than sulfate-reducing conditions (Lu et al. 2012). Experimental evidence of HMW PAHs degradation under the nitrate-reducing conditions is scarce. Only recently, some pure bacterial cultures capable of degrading HMW PAHs have been obtained. Yang et al. (2013) isolated *Pseudomonas* sp. JP1 form river sediment that can degrade BaP, fluoranthene, and phenanthrene. Liang et al. (2014) isolated a pyrene-degrading bacterium, *Paracoccus denitrificans*, from river sediment. *Cellulosimicrobium cellulans* CWS2 that has been isolated from coking plant soil could degrade BaP (Qin et al. 2018). Qin et al. (2017) isolated a BaP-degrading *Microbacterium* sp. strain from contaminated soil. It is worth noting here that some habitats such as a continental shelf, shallow lake, and wetland experience fluctuations in oxygen level on a daily or seasonal basis. Facultative anaerobes, especially NRB, might have a potential role in PAHs removal from these habitats. However, to the best of our knowledge, no study so far has investigated the role of nitrate reducers in PAHs removal from an environment that experiences fluctuating oxygen regime.

8.2 PAHs Biodegradation and Nitrate Consumption

PAHs degradation under nitrate-reducing conditions depends on the availability of nitrate to support respiration (Mihelcic and Luthy 1988a, b; Al-Bashir et al. 1990; Rockne and Strand 1998, 2001; Rockne et al. 2000). In a soil-water system, limiting nitrate concentration did not allow naphthalene and acenaphthene degradation. Only when excess nitrate was provided, degradation of the substrates commenced (Mihelcic and Luthy 1988b). Al-Bashir et al. (1990) demonstrated a linear relationship between naphthalene mineralization and nitrate depletion. Stoichiometric depletion of nitrate and degradation of PAHs was also observed by Rockne and Strand (1998). Recently, Qin et al. (2017) reported that BaP degradation by *Microbacterium* sp. was affected by C:N ratio and BaP:nitrate ratio of 1:33 resulting in 84.2% degradation in 10 days.

Nitrate demand for anaerobic oxidation (per mole of PAHs) depends on the reduction chemistry. Theoretically, one mole of naphthalene degradation requires 9.6 mol of nitrate assuming complete denitrification (Table 2) and 24 mol assuming partial reduction to nitrite (Dou et al. 2009). However, experimental values (10.71–12.02 mol) obtained by Dou et al. (2009) were in between the theoretical values. Additionally, the observed disproportion between nitrate consumption and nitrite accumulation suggested that only a fraction of nitrite is converted to dinitrogen rather than complete denitrification (Dou et al. 2009). A similar relationship between nitrate and PAHs depletion was also observed by Rockne and Strand (1998). The reaction stoichiometry of PAHs degradation and nitrate reduction is crucial for determining the extent of nitrate amendment for stimulation of biodegradation and, at the same time, avoiding adverse effects of nitrate, nitrite, and nitrogen oxides.

8.3 Enhanced PAHs Biodegradation by Nitrate Amendment

Available nitrate that initially supports the microbial degradation of organic compounds would be depleted in an environment with high contaminant load. It should be noted that total organic carbon load is also important in the context of nitrate availability in a given habitat. In such a situation, replenishing nitrate by external amendment could help to resume biodegradation. Naphthalene and phenanthrene degradation were ceased when nitrate was depleted from the media. Refeeding of the culture with nitrate re-established degradation of PAHs (Rockne and Strand 2001). Nitrate was injected into PAHs-contaminated Hamilton sediment, Canada, to enhance biodegradation. Among the 16 priority PAHs, 15 of them were degraded in the sediment (Murphy et al. 1995). Tang et al. (2005) demonstrated that slow release of nitrate from nitrocellulose in an anaerobic marine sediment increased phenanthrene degradation by 2–3 orders of magnitude.

The above findings suggest that nitrate addition to the anoxic contaminated environment may be useful for enhancing bioremediation. Nitrate amendment in PAHs-contaminated soil enhanced the abundance and activity of denitrifying bacteria and induced a shift in microbial community structure (Zhou et al. 2017). To examine the effect of nitrate addition to contaminated sediment, Xu et al. (2014) injected $Ca(NO_3)_2$ solution into the sediment of a field-scale in situ bioremediation site. They observed changes, induced by nitrate addition, in functional diversity, composition, structure, and dynamics of sediment microbial communities using GeoChip 4.0 gene array technology (Tu et al. 2014). Functional genes involved in C, N, P, and S cycling were enriched in metabolically versatile microbial members of the community. Reduced total organic carbon (TOC) as well as polybrominated diphenyl ethers and PAHs level after injection indicated that the nitrate amendment was effective in increasing potential of the sediment microflora in PAHs bioremediation. Xu et al. (2015) also reported enrichment of several aerobic PAHs-degrading genes in the nitrate-amended sediment. However, differential enrichment of genes involved in anaerobic PAHs degradation after nitrate amendment in a real contaminated environment has not been documented yet. It should be noted that the fate of supplied nitrate depends on soil chemistry, C:N ratio, total carbon load, temperature, and concentrations of nitrate-nitrite and sulfide (Tiedje et al. 1983; Akunna et al. 1993; Kraft et al. 2014). Excess of nitrate and nitrite affects biodiversity and ecosystem (Sutton et al. 2011). Therefore, the dosage of nitrate amendment to a contaminated sediment should be carefully determined to ensure considerable degradation and avoidance of excess nitrogen toxicity.

9 PAHs Biodegradation Under Iron-Reducing Conditions

Iron constitutes approximately 80% of the inner and outer cores of Earth. It is the fourth most abundant element in Earth's crust (Frey and Reed 2012). Iron-reducing bacteria participate in the anaerobic degradation of organic matter (Canfield et al. 1993), BTEX compounds (Edwards et al. 1992; Jahn et al. 2005), phenols, and *p*-cresol (Lovley and Lonergan 1990). However, very few reports on PAHs degradation under iron-reducing conditions are available. Anderson and Lovley (1999) demonstrated anaerobic naphthalene oxidation to CO_2 in petroleum-contaminated aquifer sediment where the iron reduction was the terminal electron-accepting process. An iron-reducing enrichment culture, N49, degraded naphthalene. It was enriched from a sediment sample of monitoring well set at a former MGP site (Kleemann and Meckenstock 2011). The culture is composed mainly of one bacterial member that is closely related to the significant organism in the iron-reducing, benzene-degrading enrichment culture, BF, as revealed by T-RFLP pattern and 16S rRNA gene sequences. Apart from naphthalene, N49 can also grow on 1-MN, 2-MN, 1-naphthoic acid (1-NA), or 2-NA. *Hydrogenophaga* sp. PYR1, an iron-reducing facultative anaerobe, has been recently isolated from PAHs-contaminated river sediment (Yan et al. 2017). This biosurfactant-producing bacterium degraded

both pyrene and BaP under both aerobic and iron-reducing conditions. Marozava et al. (2018) enriched a 1-MN-degrading culture from contaminated soil at a former coal gasification site using Fe(III) as the TEA. The enrichment culture consisted of two bacteria related to uncultured Gram-positive *Thermoanaerobacteraceae* and uncultured Gram-negative *Desulfobulbaceae*. The culture could also grow on naphthalene and 2-MN.

10 PAHs Biodegradation Under Sulfate-Reducing Conditions

10.1 PAHs Degradation by Sulfate-Reducing Bacteria

Sulfur is one of the most abundant elements on Earth. Sulfate ion significantly influences microbial activities in anaerobic environments (Capone and Kiene 1988). Dissimilatory sulfate reduction by anaerobic bacteria and archaea is a crucial and perhaps one of the earliest biochemical processes on Earth. SRB play a crucial role in global sulfur cycling (Muyzer and Stams 2008). SRB belong to ~23 bacterial genera representing only 7 phylogenetic lineages, 5 within bacteria (*Deltaproteobacteria*, Gram-positive *Clostridia*, *Nitrospirae*, *Thermodesulfobacteria*, and *Thermodesulfobiceae*) and 2 within archaea (Euryarchaeota and Crenarchaeota) (Muyzer and Stams 2008). During anaerobic degradation of organic matter, SRB use sulfate as the TEA and produce hydrogen sulfide in this process. SRB are widespread in freshwater and marine sediment, aquifer materials, hydrothermal vent, volcanic mud, and anaerobic sludge (Widdel and Bak 1992; Muyzer and Stams 2008). Earth's ocean is a main sink of sulfate; hence, sulfate is not a limiting nutrient in the marine environment. Thus, anaerobic degradation of organic matter in marine sediments by SRB becomes a major element cycling mechanism. Some SRB can degrade organic pollutants such as BTEX compounds (Edwards et al. 1992; Lovley et al. 1995; Phelps et al. 1996; Meckenstock et al. 2016). To date, PAHs degradation coupled with sulfate reduction has been demonstrated in many enrichments and pure cultures. Table 4 summarizes most of the available reports on PAHs degradation under sulfate-reducing conditions.

Among the PAHs, the processes of naphthalene and 2-MN degradation by SRB are better explored. Most of the information on genetics and biochemistry of anaerobic degradation of PAHs has been obtained from naphthalene and 2-MN-degrading SRB enrichments and pure cultures. SRB are abundant in sediment; so, it is not surprising that the majority of PAHs-degrading SRB cultures are obtained from freshwater and marine sediments. Coates et al. (1997) obtained naphthalene- and phenanthrene-degrading enrichment cultures from contaminated marine harbor sediments. PAHs oxidation rate was higher in the heavily contaminated sediment than that in less contaminated sediment. The former sediment

Table 4 Bacterial degradation of PAHs under sulfate-reducing conditions

Substrate	Sample/ contamination history	Contaminants	Initial PAH conc. (μM)	Inoculum	SO_4^{2-} conc. (mM)	Incubation temperature (°C)	Incubation period (day)	Degradation rate (μM day^{-1})	Reference
Ace	Petrochemical sludge	NA	6.48 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	3.78 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Ace	Municipal sewage sludge	NA	6.48 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.36 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Ant	Petrochemical sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	1.36 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Ant	Municipal sewage sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	1.8 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Flu	Swine waste-water sludge	NA	30	Bacterial culture	13.5	30	21	1.28	Tsai et al. (2009)
Flu	Petrochemical sludge	NA	6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	2.94 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Flu	Municipal sewage sludge	NA	6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	1.04 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Nap	Harbor sediment	Chemical and petrochemical	200	Sediment slurry	20	30	150	0.75	Zhang and Young (1997)
Nap	Contaminated harbor sediment	Creosote	6.50	Enrichment culture	NA	20	160	0.43 mg (g of VSS $day)^{-1}$	Rockne and Strand (1998)
Nap	Groundwater; creosote-based wood preser-vation facility	PAHs (up to 640 mg kg^{-1})	7,800	Enrichment culture SobN1	20	20	~600	13.33	Kummel et al. (2015)

Nap	Groundwater sample from aquifer; gas production by coal carbonization and hydrocarbon cracking	PAHs (up to 280 mg kg^{-1} sediment, up to 4.6 mg BTEX L^{-1} groundwater	7,800	Enrichment culture MicN1	20	20	~600	13.33	Kummel et al. (2015)
Nap	Sediment; brown coal processing	Up to 35 mg PAHs kg^{-1} sediment, up to 13 mg BTEX L^{-1} groundwater	7,800	Enrichment culture GölN1	20	20	~600	7	Kummel et al. (2015)
Nap	Groundwater; fuel depot	Up to 0.8 mg PAHs L^{-1} groundwater, up to 11 mg BTEX L^{-1} groundwater	7,800	Enrichment culture EgN1	20	20	~600	7.27	Kummel et al. (2015)
Nap	Sediment; fuel depot	Up to 0.8 mg PAHs L^{-1} groundwater, up to 11 mg BTEX L^{-1} groundwater	7,800	Enrichment culture EgN2	20	20	~600	13.33	Kummel et al. (2015)
Nap	Mediterranean lagoon sediment	NA	156.04	Strain NaphS3	NA	NA	NA	NA	Musat et al. (2009)
Nap	Mediterranean lagoon sediment	NA	156.04	Strain NaphS6	NA	NA	NA	NA	Musat et al. (2009)
Nap	North Sea harbor sediment	NA	64.40	Strain NaphS2	NA	28–30	~60	2.14	Galushko et al. (1999)

(continued)

Table 4 (continued)

Substrate	Sample/ contamination history	Contaminants	Initial PAH conc. (μM)	Inoculum	SO_4^{2-} conc. (mM)	Incubation temperature (°C)	Incubation period (day)	Degradation rate (μM day^{-1})	Reference
Nap	Soil material from contaminated aquifer	NA	50.00	Soil	28.125	NA	NA	NA	Meckenstock et al. (2000)
Nap	Sediment from coal tar contaminated aquifer	NA	5.5–8.5	Sediment microcosm	20	25	9	4.76–6.12	Meckenstock et al. (2016)
Nap	Contaminated soil and river sediment	Benzene, toluene and PAHs	25[a]	As is	10	20	425	70–80% removal after 100 days	Langenhoff et al. (1996)
Nap[b]	Contaminated bay sediment	Hydrocarbons, 33 mg kg^{-1} PAHs	1 μCi	Sediment enrichment	10	25	62	82% radiolabel recovery as $^{14}CO_2$	Coates et al. (1996)
Nap	Soil material of a contaminated aquifer	NA	Liquid or crystal 2–4 mg 50 mL^{-1}	Enrichment culture	10	NA	NA	35.71 μM sulfide day^{-1}	Meckenstock et al. (2000)
Phe	Swine waste water sludge	NA	28.00	Bacterial culture	13.5	30	21	0.80	Tsai et al. (2009)
Phe	Petrochemical sludge	NA	5.6	Contaminated river sediment enrichment	20	30	NA	1.76	Chang et al. (2003)
Phe	Municipal sewage sludge	NA	5.6	Contaminated river sediment enrichment	20	30	NA	3.61	Chang et al. (2003)
Phe	Creosote-contaminated harbor sediment	Creosote	4.50	Enrichment culture	NA	20	135	0.12 mg (g of VSS $day)^{-1}$	Rockne and Strand (1998)
Phe[b]	Contaminated bay sediment	Hydrocarbons, 33 mg kg^{-1} PAHs	1 μCi	Sediment enrichment	10	26	60	99% radiolabel recovery as $^{14}CO_2$	Coates et al. (1996)

Phe	Hydrocarbon-contaminated marine sediment	Hydrocarbon	NA	Sediment-free enrichment culture	28 and 33 weeks[c]	28	–	23.1 ± 1.15 μmol Phe; 20–45% loss of ^{14}C-Phe	Davidova et al. (2007)
Pyr	Contaminated harbor sediment	Municipal sewage and industrial effluent	100 mg kg^{-1}	Sediment enrichment	NA	25	30	1.33 mg kg^{-1} day^{-1}	Lu et al. (2012)
Pyr	Petrochemical sludge	NA	4.9 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.69 μmol kg^{-1} day^{-1}	Chang et al. (2003)
Pyr	Municipal sewage sludge	NA	4.9 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	1.16 μmol kg^{-1} day^{-1}	Chang et al. (2003)
2-MNap	Mediterranean lagoon sediment		70.32	Strain NaphS6	NA	NA	NA	NA	Musat et al. (2009)
2-MNap	Soil from contaminated aquifer	NA	NA	NA	NA	NA	NA	NA	Annweiler et al. (2000)

Ace acenaphthene, *Ant* anthracene, *Flu* fluorene, *Nap* naphthalene, *Phe* phenanthrene, *Pyr* pyrene, *2-MNap* 2-methylnaphthalene, *NA* not available

[a]Continuous flow of 25 μM naphthalene

[b]Radiolabeled substrate

[c]28 weeks for radiolabeled substrate degradation experiment and 33 weeks for nonlabeled substrate

enrichment could also degrade methylnaphthalene, fluorene, and fluoranthene under sulfate-reducing conditions. Bedessem et al. (1997) established several sulfate-reducing naphthalene-degrading enrichment cultures from creosote-contaminated aquifer sediment and maintained them throughout for 3 years. After repeated feeding with naphthalene, the duration of initial lag (1–20 weeks) was reduced to a minimum, and the adapted enrichment could mineralize 66% of added ^{14}C-naphthalene to $^{14}CO_2$ in 13 days. Zhang and Young (1997) enriched naphthalene- and phenanthrene-degrading culture from contaminated harbor sediment under strict sulfate-reducing conditions. The cultures could degrade 150–200 μM naphthalene and phenanthrene within 150 days. N47, which is one of the thoroughly investigated enrichment cultures, was derived from the soil of a contaminated aquifer near Stuttgart, Germany (Meckenstock et al. 2000). The culture utilized naphthalene and 2-MN without a significant lag (Annweiler et al. 2000; Meckenstock et al. 2000). Thus, N47 is one of the few anaerobic PAHs-degrading cultures that can consistently degrade naphthalene and 2-MN upon repeated transfer. Terminal fragment length analysis and 16S rRNA gene sequencing of N47 revealed that the culture is composed of an unidentified member of *Deltaproteobacteria* in association with 7% of *Spirochaetes* members. In addition to naphthalene and 2-MN, N47 can also co-metabolically degrade different poly- and heterocyclic aromatic hydrocarbon compounds (Safinowski et al. 2006). Rothermich et al. (2002) demonstrated mineralization of ^{14}C-naphthalene and ^{14}C-phenanthrene in sulfidogenic contaminated harbor sediment.

Moreover, degradation of in situ PAHs pool (naphthalene, 1-MN, 2-MN, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, and BaP) was demonstrated in sediment microcosm (Safinowski et al. 2006). In this study, LMW PAHs except naphthalene were degraded more rapidly than HMW congeners. Davidova et al. (2007) enriched a phenanthrene-degrading culture from hydrocarbon-contaminated marine sediment. The culture is mainly composed of members of *Deltaproteobacteria* that are like other known hydrocarbon degraders and uncultured clones obtained from hydrocarbon-degrading communities. An SRB enrichment culture was obtained from swine sewage sludge that can degrade fluorene and phenanthrene (Tsai et al. 2009). The enrichment degraded 88% fluorene and 65% phenanthrene (initially 5 mg L^{-1} each) after 21 days of operation. However, when the substrate mixture was provided as a carbon source, the degradation rate decreased, indicating that an enrichment or a pure culture may degrade a single compound more efficiently than mixed substrates. Environmental contaminants exist as conglomeration and degradation efficiency of culture in microcosm might not necessarily be the same as in real contaminated site. An SRB pure culture, NaphS2, isolated from North Sea harbor sediment, degraded naphthalene and 2-MN under sulfate reduction conditions (Galushko et al. 1999). Two other pure strains, NaphS3 and NaphS6, were isolated from Mediterranean lagoon sediment using naphthalene as the substrate (Musat et al. 2009). Both strains also utilized 2-MN as the sole carbon source. All the pure bacterial strains (NaphS2, NaphS3, and NaphS6) are affiliated with δ-subclass of *Proteobacteria* and are closely related to SRB that

degrade some other aromatic compounds. Five naphthalene-degrading SRB enrichment cultures (SobN1, MicN1, GölN1, EgN1, and EgN2) were enriched from contaminated groundwater and aquifer sediment (Kummel et al. 2015). These highly enriched cultures degraded naphthalene at a much higher concentration (7.8 mM) with appreciable degradation rates (7–13.3 μM day^{-1}). Acenaphthene and phenanthrene degradation ability of the enrichment cultures was tested, but none of the cultures could utilize the PAHs. Very recently, Himmelberg et al. (2018) enriched phenanthrene-degrading and sulfate-reducing culture, namely, TRIP1, from muddy soil mixture sample in which a member of *Desulfobacteraceae* that was very closely related to naphthalene-degrading strain, NaphS2, dominated in the enrichment.

The coupling of sulfate reduction with PAHs degradation has been demonstrated in many sulfate-reducing enrichment cultures. Partial or complete inhibition of PAHs degradation upon the addition of sulfate reduction inhibitor is a hallmark of direct involvement of SRB in PAHs degradation process. Several studies used sodium molybdate (usually 20 mM) as sulfate reduction inhibitor to demonstrate the role of SRB in PAHs degradation (Coates et al. 1996; Bedessem et al. 1997; Annweiler et al. 2000; Meckenstock et al. 2000; Rothermich et al. 2002; Davidova et al. 2007). Besides, stoichiometric sulfate loss from the media assuming complete mineralization of substrate PAHs was also demonstrated (Davidova et al. 2007). Furthermore, the reduction of ^{35}S-sulfate to ^{35}S-sulfide during naphthalene degradation by a sulfate-reducing enrichment microcosm was demonstrated by Bedessem et al. (1997). Although anaerobic degradation of some PAHs, exceptionally low-molecular-weight congeners, under sulfate reduction condition is better investigated and biochemical mechanisms of naphthalene and 2-MN degradation have been elucidated, similar investigations on HMW PAHs such as pyrene, BaP, or chrysene are rarely reported. Pyrene degradation under sulfate-reducing conditions has been reported in some instances. However, no culture capable of consistently degrading pyrene has been identified.

10.2 Enhanced PAHs Biodegradation by Sulfate Amendment

Although sulfate is abundant in seawater, availability of sulfate in anoxic sediment depends on diffusion of seawater sulfate to sediment. Moreover, utilization of sulfate during anaerobic oxidation of organic substances may exceed sulfate supply from overlaying sulfate-rich water (Martens and Val Klump 1984). On the other hand, freshwater sediments are generally low in sulfate content compared to marine sediments. In general, sulfate reduction will predominate over methanogenesis if sulfate supply is enough to sustain the process (Muyzer and Stams 2008). In both fresh and marine water systems, when sedimentary sulfate concentration decreases because of the extensive activity of SRB or other physicochemical reasons, methanogenic degradation becomes the primary means of organic compound decomposition. Methanogenesis, however, is a less efficient energy-generating process than sulfate reduction. In sulfate-depleted sediment, the external amendment

could help to restore SRB-mediated bioremediation. Sulfate addition has been shown to be effective in stimulating anaerobic benzene (Weiner et al. 1998; Anderson and Lovley 2000) and BTEX (Cunningham et al. 2001) degradation.

The addition of sulfate in the form of soluble sodium sulfate and less soluble gypsum to a sulfate-depleted methanogenic sediment stimulated naphthalene and 2-MN degradation (Rothermich et al. 2002). Similarly, the controlled release of sulfate in marine sediment significantly enhanced anaerobic degradation of phenanthrene (Tang et al. 2005). Sulfate addition in more soluble form may be practicable for enhancing bioremediation potential of contaminated groundwater and aquifer sediment, whereas addition of less soluble form (e.g., gypsum) may be useful in alleviating sulfate concentration in the marine system (Rothermich et al. 2002). Nonetheless, Bach et al. (2005) did not find any stimulatory effect of sulfate addition in a sulfate-deficient PAHs-contaminated estuarine sediment. To date, the effect of sulfate amendment on shaping sediment microbial community structure and function and in situ demonstrations of the stimulatory effect of sulfate in PAHs degradation have not been reported.

11 PAHs Biodegradation Under Methanogenic Conditions

PAHs degradation under methanogenic conditions is a thermodynamically challenging process, as the net gain of ATP during the biodegradation process is extremely low (Dolfing et al. 2009). Complex organic compounds such as polysaccharides, halogenated organic compounds, alkanes, as well as PAHs can be transformed via syntrophic metabolism which involves cross-feeding between microbial species (Schink 1997). In anaerobic syntrophic metabolism, substrates are first hydrolyzed to acetate, longer-chain fatty acids, propionate, alcohols, CO_2, formate, and H_2 by fermentative bacteria. Subsequently, the other crucial participant of a syntrophic consortium, methanogenic bacteria, utilizes some of the products of the preceding fermentative metabolism to reduce CO_2 to CH_4. The conversion of the most oxidized state of carbon, i.e., CO_2, to the most reduced form, i.e., CH_4, generates adequate energy that makes the overall anaerobic transformation process thermodynamically feasible (McInerney et al. 2009). Christensen et al. (2004) inferred from a thermodynamic calculation that methanogenic degradation of naphthalene is feasible only in the presence of hydrogen-utilizing methanogens. PAHs are widespread in fuel-contaminated methanogenic sediments, oil reservoirs, and groundwater aquifers. Methanogenic degradation of PAHs can be a major route of PAHs detoxification in methane-rich environments. So far, degradation of some LMW PAHs under methanogenic conditions has been reported in contaminated soils, sewage and petrochemical sludge, and sediments (Table 5). However, the degradability of HMW PAHs under methanogenic conditions remains elusive due to the dearth of experimental evidence. Methanogenic degradation of PAHs with two or more rings was demonstrated in contaminated sewage sludge (Trably et al. 2003; Christensen et al. 2004; Cea-Barcia et al. 2013). Zhang et al. (2012b) demonstrated incorporation

Table 5 PAHs degradation under methanogenic conditions

Substrate	Origin of sample	Contaminants	Initial PAHs conc.	Inoculum	TEA conc. (mM)	Incubation temperature (°C)	Incubation period (day)	Degradation rate (μM day^{-1})	Reference
Ace	Petrochemical sludge	NA	6.48 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	3.13	Chang et al. (2003)
Ace	Municipal sewage sludge	NA	6.48 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.32	Chang et al. (2003)
Ant	Petrochemical sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.7	Chang et al. (2003)
Ant	Municipal sewage sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.68	Chang et al. (2003)
Ant	Shallow aquifer sediment sample	20–30 μg ant	200 μg ant per 10 ml media and 3 g sediment	Sediment enrichment culture	–	20	120	1.35	Wan et al. (2012)
Flu	Contaminated subsurface mangrove swamp sediment	PAHs 5 μg/g	10 μg g^{-1}	Enriched bacteria	20	28	125	0.08	Li et al. (2015)
Flu	Petrochemical sludge	NA	6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	2.24	Chang et al. (2003)
Flu	Municipal sewage sludge	NA	6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.64	Chang et al. (2003)
Fluoran	Contaminated subsurface mangrove swamp sediment	PAHs 5 μg/g	10 μg g^{-1}	Enriched bacteria	20	28	125	0.06	Li et al. (2015)

(continued)

Table 5 (continued)

Substrate	Origin of sample	Contaminants	Initial PAHs conc.	Inoculum	TEA conc. (mM)	Incubation temperature (°C)	Incubation period (day)	Degradation rate (μM day^{-1})	Reference
Nap	Contaminated sub-surface sediment	Natural gas condensate	58.5 mM	Enrichment culture	–	30	320	1.24 μmol CH_4 day^{-1}	Toth et al. (2018)
Nap	Harbor sediment	NA	200 μM	Sediment enrichment culture	–	30	~130	0.88	Chang et al. (2006)
Phe	Contaminated sub-surface mangrove swamp sediment	PAHs 5 μg/g	10 μg g^{-1}	Enriched bacteria	20	28	125	0.08	Li et al. (2015)
Phe	Petrochemical sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	1.35	Chang et al. (2003)
Phe	Municipal sewage sludge	NA	5.6 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	2.46	Chang et al. (2003)
Phe	Harbor sediment	NA	200 μM	Sediment enrichment culture	–	30	~130	0.95	Chang et al. (2006)
Pyr	Contaminated sub-surface mangrove swamp sediment	PAHs 5 μg/g	10 μg g^{-1}	Enriched bacteria	20	28	125	0.05	Li et al. (2015)
Pyr	Petrochemical sludge	NA	4.9 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	0.1	Chang et al. (2003)
Pyr	Municipal sewage sludge	NA	4.9 μmol kg^{-1}	Contaminated river sediment enrichment	20	30	NA	1.05	Chang et al. (2003)

2,6-DiMNap	Contaminated sub-surface sediment	Natural gas condensate	51.20 µM	Enrichment culture	–	21–23	110	3.52 µmol CH_4 day^{-1}	Berdugo-Clavijo et al. (2012)
2-MNap	Contaminated sub-surface sediment	Natural gas condensate	56.30 µM	Enrichment culture	–	21–23	110	3.83 µmol CH_4 day^{-1}	Berdugo-Clavijo et al. (2012)

Ace acenaphthene, *Ant* anthracene, *Flu* fluorene, *Fluoran* fluoranthene, *Nap* naphthalene, *Phe* phenanthrene, *Pyr* pyrene, *NA* not available

of labeled carbon from $^{13}C_6$-anthracene in the microbial community of landfill leachate-contaminated subsurface aquifer sediment under methanogenic conditions. Production of methane relative to the sterile control in this study also suggested methanogenic degradation of anthracene. In another study, naphthalene- and phenanthrene-degrading methanogenic enrichment cultures were developed with Baltimore Harbor sediments without the addition of any external electron acceptors (Chang et al. 2001). As PAHs are natural components of crude oil, microorganisms that can degrade alkane compounds may also adapt to and degrade aromatic hydrocarbons. Berdugo-Clavijo et al. (2012) examined the ability of a methanogenic enrichment culture in biodegradation of naphthalene and methylated naphthalene substitutes. The methanogenic enrichment was obtained from aquifer sediment that was contaminated with natural gas compensate. The culture was able to degrade alkane fraction of crude oil with concomitant production of methane (Townsend et al. 2003; Gieg et al. 2008). Under methanogenic conditions, 2-MN- and 2,6-dimethylnaphthalene-amended enrichments produced methane gas compared to unamended controls. However, naphthalene- and 1-MN-amended culture did not show substantial amount of methane production. Interestingly, Toth et al. (2018) demonstrated development of naphthalene degradation ability of the methanogenic culture after long adaptation period. The findings suggested that methanogenic microorganisms in crude oil-contaminated sediment could adapt to PAHs and, over time, developed considerable degradation ability.

The involvement of methanogens in PAHs degradation has been demonstrated experimentally. In PAHs-degrading methanogenic enrichment cultures, the addition of methanogenesis inhibitor, bromoethane sulfonic acid (BESA), and eubacterial inhibitor, vancomycin, caused a significant reduction in degradation rates indicating the role of both methanogens and syntrophic bacteria (Chang et al. 2003). Similarly, the addition of BESA to a naphthalene- and phenanthrene-degrading consortia partially inhibited degradation and eliminated archaeal members from the consortia suggesting the involvement of methanogens in the degradation process (Chang et al. 2006). Fluorescence in situ hybridization analysis of naphthalene- and 1-MN-degrading enriched methanogenic consortia revealed that both bacteria and archaea were involved in the degradation process. Moreover, the presence of the members of *Methanobacteriales* in the consortia indicated the involvement of syntrophic obligate hydrogen- or formate-utilizing archaea (Christensen et al. 2004).

Several members of methanogenic PAHs-degrading community have been identified. Microbial community analysis revealed the dominance of archaeal members affiliated with *Methanosaeta* and *Methanoculleus* species and bacterial members related to the *Clostridiaceae* in aquifer sediment enrichments that degrade 2-MN and 2,6-dimethylnaphthalene (Berdugo-Clavijo et al. 2012). The cultures were previously reported incapable of degrading naphthalene under methanogenic conditions. However, after repeated transfer and tuning of the culture conditions, an enrichment culture capable of mineralizing naphthalene to methane has been obtained recently (Toth et al. 2018). Using next-generation sequencing and DNA-stable isotope probing techniques, the authors identified an unclassified *Clostridiaceae* species as a putative naphthalene degrader along with a *Desulfuromonadales* phylotype

(function unknown). In another study, clone library analysis of phenanthrene-degrading leachate-contaminated sediment microcosm identified a community consisting of *γ-Proteobacteria* dominated by members of *Citrobacter* and *Pseudomonas* and archaea represented by members of *Methanosarcina*, *Methanobacterium*, and *Thermogymnomonas* (Zhang et al. 2012a). Using $^{13}C_6$-anthracene and DNA-based stable isotope probing technique, Zhang et al. (2012b) demonstrated incorporation of radiolabel into three *Proteobacteria* phylotypes represented by the genera *Methylibium* and *Legionella* and an unclassified *Rhizobiales.* Methanogenic PAHs degradation and biogas/methane production may be correlated. Degradation of PAHs in laboratory-scale stirred-tank sewage sludge digester resulted in decreased biogas production but increased PAHs removal (Trably et al. 2003). In contrast, near stoichiometric production of methane was reported in methanogenic enrichment culture amended with 2-MN and 2, 6-dimethylnaphthalene (Berdugo-Clavijo et al. 2012).

12 Anaerobic PAHs Degradation by Pure Cultures of Bacteria

Pure cultures are invaluable especially in investigating the molecular mechanism of biodegradation. Several pure cultures of bacteria capable of degrading PAHs anaerobically have been isolated (Table 6). Among them, only three are strictly anaerobic sulfate-reducing bacterial strains, whereas most of the isolates are facultative anaerobic nitrate reducers. The scarcity of strictly anaerobic PAHs-degrading pure isolates may be due to (a) complex nutrient requirement, (b) slow growth, (c) failure to provide in situ-like incubation environment, and (d) obligatory mutualistic dependence among members of degrading consortium. Attempts to isolate *Deltaproteobacterium* sp. from naphthalene-degrading enrichment culture, N47, failed even though most of the genes/proteins involved in degradation are associated with this bacterium (Safinowski and Meckenstock 2006; Selesi et al. 2010; Bergmann et al. 2011b). In another case, Martirani-Von Abercron et al. (2016) isolated several naphthalene-degrading and nitrate-reducing bacterial strains. However, nitrate-respiring and naphthalene-degrading properties of the pure isolates could not be reproduced in liquid culture. A naphthalene-degrading pure culture, NaphS2, was isolated from the anoxic black sediment of North Sea (Galushko et al. 1999). Two other naphthalene-degrading SRB cultures, NaphS3 and NaphS6, were obtained from anoxic Mediterranean lagoon sediment (Musat et al. 2009). Much information on anaerobic naphthalene degradation has been obtained from *Deltaproteobacteria* NaphS2. Rockne et al. (2000) isolated three nitrate-reducing naphthalene-degrading facultative anaerobic bacteria. Stain NAP-3-1 and NAP-4 are phylogenetically close to *Pseudomonas stutzeri* and *Vibrio pelagius*, respectively. McNally et al. (1998) demonstrated anthracene, phenanthrene, and pyrene degradation by nitrate-reducing facultative anaerobic bacteria, *Pseudomonas stutzeri*

Table 6 Pure cultures of bacteria capable of degrading PAHs anaerobically

Bacterium	Obligate/ facultative anaerobe	Substrate	Origin	TEA	Reference
Hydrogenophaga sp. PYR1	Facultative anaerobe	Pyrene and BaP	Contaminated sediment	Facultative anaerobe/ iron(III)	Yan et al. (2017)
Microbacterium sp.	Facultative anaerobe	BaP	Contaminated soils	Nitrate	Qin et al. (2017)
Pseudomonas aeruginosa PAH-1	Facultative anaerobe	Phenanthrene	Anodic solution from a microbial fuel cell	AQDS/humus	Ma et al. (2011)
Deltaproteobacteria NaphS2	Obligate anaerobe	Naphthalene and 2-MN	North Sea harbor sediment	Sulfate	Galushko et al. (1999)
Deltaproteobacteria NaphS3	Obligate anaerobe	Naphthalene and 2-MN	Black (anoxic) sediment from a Mediterranean lagoon	Sulfate	Musat et al. (2009)
Deltaproteobacteria NaphS6	Obligate anaerobe	Naphthalene and 2-MN	Black (anoxic) sediment from a Mediterranean lagoon	Sulfate	Musat et al. (2009)
Paracoccus denitrificans	Facultative anaerobe	Pyrene	River sediment	Nitrate/nitrite	Yang et al. (2013)
Vibrio pelagius NAP-4	Facultative anaerobe	Naphthalene	Fluidized bed reactor enrichment culture seeded with PAHs-contaminated marine sediment	Nitrate	Rockne et al. (2000)
Pseudomonas stutzeri NAP-3-1	Facultative anaerobe	Naphthalene	Fluidized bed reactor enrichment culture seeded with PAHs-contaminated marine sediment	Nitrate	Rockne et al. (2000)
Pseudomonas sp. JP1	Facultative anaerobe	BaP, fluoranthene, and phenanthrene	River sediment	Nitrate	Liang et al. (2014)
P. stutzeri SAG-R	Facultative anaerobe	Anthracene, phenanthrene, and pyrene	Soil obtained from a creosote contaminated hazardous waste site	Nitrate	McNally et al. (1998)
Pseudomonas fluorescens W-2	Facultative anaerobe	Anthracene, phenanthrene, and pyrene	Soil obtained from a creosote contaminated hazardous waste site	Nitrate	McNally et al. (1998)
Pseudomonas putida KBM-1	Facultative anaerobe	Anthracene, phenanthrene, and pyrene	Bay sediment	Nitrate	McNally et al. (1998)

SAG-R, *P. fluorescens* W-2, and *P. putida* KBM-1. Recently, *Microbacterium* sp. (Qin et al. 2017) and *Cellulosimicrobium cellulans* CWS2 (Qin et al. 2018) have been shown to degrade BaP under nitrate-reducing conditions. The efficiency of pure cultures over enrichment cultures in anaerobic PAHs degradation is not fully understood. Pure strains, NAP-3-1 and NAP-4, were less efficient in degrading naphthalene compared to their efficiency in the consortia; better degradation in co-culture may be due to the consortium synergy (Rockne et al. 2000). Pure cultures may not necessarily reflect the bioremediation potency of the environment from which they are obtained. Nevertheless, the value of obtaining pure bacterial strains for the elucidation of the molecular mechanism of degradation remains crucial.

13 Effects of PAHs Contamination on Anaerobic Microbial Community

PAHs input in an environment changes the total organic matter content. Depending on solubility and bioavailability of contaminants, an alteration in dissolved and particulate organic matter content is expected. The altered ratio, together with the toxicity of PAHs, would have a consequence on the natural microbial community. Knowledge on PAHs-induced changes in the microbial community is almost vague, as only a few studies have been conducted so far. Thus, PAHs-induced selection pressure on the microbial community is evident from the available few instances of evidence. Chang et al. (2005) investigated the effect of naphthalene and phenanthrene on methanogenic microbial community in harbor sediment. They found that SRB dominated the baseline community. Addition of naphthalene or phenanthrene triggered a marked shift toward the enrichment of methanogenic community in that sediment.

Comparative analysis of 16S rRNA genes indicated that naphthalene- and phenanthrene-degrading communities were different. Wan et al. (2012) reported an increased abundance of archaeal community in anthracene-treated methanogenic sediment. Alejandro et al. (2013) investigated the impact of *Prestige* oil spill on the microbial community in contaminated subtidal sediment after 18 and 53 months of the spill. The spilled oil contained naphthalene and its methylated derivatives. Along the depth, aerobic hydrocarbon degraders were abundant in the upper zone, NRB were present in higher number in the oxidized zone, and their number decreased with depth while SRB count reached a maximum at depth of 12–15 cm. The deep anoxic sediment was dominated by strictly anaerobic SRB. Although the spill caused a decrease in bacterial count, aromatic-oxidizing cultivable populations increased with time. The time-dependent difference in community distribution was also evident. *Gammaproteobacteria* and *Deltaproteobacteria* were the dominant phyla in the contaminated sediment as indicated by 16S rRNA gene analysis and fluorescent in situ hybridization analysis. Within the *Deltaproteobacteria*, *Desulfobacteraceae* was the most abundant, and *Desulfarculales* constituted half of the specific

sequences. This study indicates that introduction of crude oil PAHs in anaerobic sediment exerts some toxic effect to the microbial community; however, resistant bacterial community will eventually develop and participate in PAHs cleaning from the impacted environment. Recently, Martirani-Von Abercron et al. (2016) investigated the effect of PAHs on different environmental samples under nitrate-reducing conditions. The samples were collected from the diverse environment: rice paddy fields, activated sludge, compost pile, lagoon sediment, and marine sediment. MPN enumeration indicated the presence of nitrate reducers and naphthalene, 2-MN, 2-NA, and anthracene degraders in all samples. Bacterial community analysis of the non-spiked control samples indicated that the samples, with one exception, were dominated by *Proteobacteria* followed by a different proportion of *Bacteroidetes*, *Chloroflexi*, and *Actinobacteria*. After enriching the samples in naphthalene and 2-MN, significant changes in the bacterial community occurred. Groups of uncultured and poorly characterized *Acidobacteria*, *Firmicutes*, and *Verrucomicrobia* could be enriched over *Proteobacteria*.

A recent study showed rapid shaping of the methanogenic microbiome in full-scale anaerobic digester reactors amended with naphthalene, fluorene, anthracene, phenanthrene, and fluoranthene (Oko et al. 2017). The adaptation was more rapid with oil and gas processing wastewater treatment reactor sludge (OG) than with municipal solid waste reactor sludge (MS). Over time, PAHs, feed, and nutrient-dependent succession in the bacterial and archaeal community was observed. After 14 days of incubation with PAHs, the relative abundance of the *Euryarchaeota* group increased by 35 and 90% in MS and OG communities, respectively, while the total abundance of bacteria decreased. In contrast, Ribeiro et al. (2018) argued that the microbial community structure in sediment microcosms amended with naphthalene and fluoranthene was mainly shaped by sample type and incubation time rather than the PAHs. In addition, Zhou et al. (2017) observed that pyrene amendment did not affect the activity and abundance of soil denitrifiers as well as microbial respiration. Also, pyrene addition to soil did not cause any change in microbial community.

14 Factors Affecting Anaerobic Biodegradation of PAHs

Many factors affect bioavailability and degradability of PAHs in the environment. In addition to the physicochemical properties of PAHs, some characteristics of the contaminated sites such as organic matter content, soil/sediment texture, clay minerals, pH, temperature, salinity, nutrient availability, and redox potential affect biodegradation of PAHs (Wilson and Jones 1993). Apart from these factors, constituents of a contaminating PAHs mixture and presence of co-contaminants such as heavy metals, cyanides, and other organic compounds also affect the microbial degradation of PAHs (Kuppusamy et al. 2017). As described in Sects. 8.3 and 10.2, the availability of TEAs such as nitrate and sulfate is a prominent factor affecting anaerobic degradation. The field of anaerobic PAHs degradation is nascent;

very few studies so far have investigated the role of factors in determining anaerobic degradation of PAHs.

14.1 Influence of Structural Complexity of PAHs on Biodegradability

In general, structurally simple compounds are better biodegradable than complex compounds. Xenobiotic compounds are comparatively persistent to biodegradation. Several rules for predicting biodegradability of natural and synthetic compounds based on structural and chemical properties have been proposed, but numerous exceptions make them overly generalized (Kobayashi and Rittmann 1982). For example, benzene is structurally simpler and show more water solubility than naphthalene. Even so, it is chemically more stable than naphthalene. Benzene remained persistent in anaerobic sediment columns while naphthalene was degraded (Langenhoff et al. 1996). Hypothetically, LMW PAHs, as they are less complex in structure and more soluble in water, should be more readily degradable than their HMW congeners. Indeed, this premise is supported by many studies. For instance, Rothermich et al. (2002) observed quicker removal of 2–3 ring PAHs than 4–5 ring congeners. In a nitrate-reducing marine sediment enrichment, LMW PAHs (acenaphthene, phenanthrene, and fluorine) were degraded more efficiently than heavier fluoranthene and pyrene; the more complex PAHs like chrysene and benz (a)anthracene were degraded very slowly, and BaP remained persistent (MacRae and Hall 1998). Naphthalene was better degraded than phenanthrene by a denitrifying fluidized bed enrichment culture (Rockne and Strand 1998). Ambrosoli et al. (2005) reported a trend of three PAHs in the following order: fluorene > phenanthrene > pyrene. In a contaminated arctic soil, naphthalene and 2-methylnaphthalene were degraded under nitrate-reducing conditions, but HMW PAHs showed persistence for 90 days (Eriksson et al. 2003). However, complexity and solubility are not the only factors that determine degradability of PAHs. In a sediment enrichment, naphthalene degradation was slow compared to other 2–5 ring PAHs tested, including BaP (Rothermich et al. 2002). Relatively complex peri-fused pyrene was better degraded than cata-condensed anthracene by denitrifying strain KBM-1 (McNally et al. 1998). Three-ring phenanthrene was degraded slowly compared to five-ring BaP by a facultative anaerobe, *Pseudomonas* sp. JP1 (Liang et al. 2014). Interestingly, Murphy et al. (1995) demonstrated equal degradation rate of 15 priority PAHs in real contaminated sediment upon nitrate supplement. It is thus apparent that a simple correlation between structural complexity and biodegradability during anaerobic PAHs degradation cannot be drawn conclusively.

It may seem that increasing concentration of PAHs in an environment could impede their degradability. Available evidence is contrasting enough that does not allow to arrive at a general conclusion. Al-Bashir et al. (1990) reported that denitrifying soil slurry enrichment culture could mineralize naphthalene at

aqueous-phase saturation level (50 ppm) and beyond (200 and 500 ppm) at the same rate. However, at concentration beyond the saturation level, degradation rate plummet after substrate concentration decreased to aqueous-phase solubility limit. The authors suggested that desorption of substrate rather than concentration determines the degradability. Dou et al. (2009) determined the degradation rates of naphthalene at a different initial concentration by mixed bacterial culture. Degradation rate increased with the increase in naphthalene concentration in 5–30 mg L^{-1} range. A BaP-degrading pure culture, *Cellulosimicrobium cellulans* CWS2, degraded 10 mg L^{-1} of BaP more efficiently than 5 mg L^{-1} concentration (Qin et al. 2018). Zhou et al. (2017) reported that increase in concentration of pyrene from 30 to 60 mg L^{-1} cause an increase in pyrene removal efficiency in denitrifying soil microcosms. Nevertheless, it can be inferred that apart from structural complexity and concentration of the contaminants, some yet unknown factors also affect degradability of PAHs in anaerobic environment.

14.2 Effect of Prior Exposure to PAHs

Experimental evidence does not allow reaching a generalized assumption that a site with previous contamination history possesses higher bioremediation potency than a pristine one. It is tempting to assume that microorganisms in a long-term contaminated site are better adapted to the pollutant(s); hence more efficient in degrading contaminants. Indeed, PAHs-degrading enrichment and pure cultures have been frequently isolated from contaminated samples. In such habitats, metabolically versatile microorganisms that can survive and utilize otherwise toxic pollutants confront selection pressure and might get competitive advantages over other community members. However, bacterial degradation of PAHs in pristine environments has also been reported in many studies. One possible explanation for the scenario is the possession of uninduced genetic machinery within the microbial members of an uncontaminated environment. Those unexpressed metabolic "toolboxes" are possibly induced after exposure to pollutants. Additionally, not all community members may have the ability to initialize attacking the pollutants. In the natural environment, many compounds including toxic contaminants are degraded co-metabolically (Hazen 2010).

Comparison between anaerobic PAHs removal efficiency of contaminated and uncontaminated (or less contaminated) indicates that samples with previous exposure history sometimes have greater bioremediation potential. Naphthalene and phenanthrene degradation in two sulfidogenic marine sediments that differ in the degree of contamination history was investigated (Coates et al. 1996). Both the substrates were readily degraded in one sediment that was severely contaminated with PAHs (33 mg of PAHs kg^{-1} of sediment). In contrast, the less contaminated sediment (4 mg PAHs kg^{-1}) showed very minimal degradation. Interestingly, naphthalene degradation ability of the less contaminated sediment was stimulated when it was incubated with some of the heavily contaminated sediment (Coates et al.

1997). Hayes et al. (1999) observed a similar correspondence between the exposure level of a sample and its PAHs removal potential. In both studies, long-term exposure of the pristine-like samples to PAHs also resulted in the development of better degradation capacity. Eriksson et al. (2003) observed a better degradation of naphthalene, 2-MN, fluorene, and phenanthrene in a nitrate-reducing arctic soil contaminated with fuel.

Previous exposure, however, does not always lead to the development of better remediation capacity. Al-Bashir et al. (1990) observed that prior exposure did not help in improving PAHs degradation rates and reduction of the lag phase. The denitrifying *Pseudomonas stutzeri* strain SAG-R was isolated from a soil of creosote-contaminated hazardous waste site. It could degrade anthracene, phenanthrene, and pyrene more efficiently than the other two bacteria tested (McNally et al. 1998). Surprisingly, one of the strains, *Pseudomonas putida* KBM-1, which was isolated from almost pristine soil, could also degrade all the three PAHs at the rates comparable to those of the strain SAG-R. Sometimes, prolonged exposure to PAHs promotes the abundance of specialized degrading microbial community that is reflected in enhanced biodegradation rate, but such contamination history is not a prerequisite in all cases.

14.3 Effect of Soil Amendments and Heavy Metals

The effects of nitrate and sulfate amendment in enhancing natural attenuation of PAHs in an anaerobic environment and changes in abundance, activity, and community structure following the addition of inorganic electron acceptors have already been discussed. Li et al. (2015) investigated the effect of bicarbonate addition on anaerobic degradation of fluorene, phenanthrene, fluoranthene, and pyrene. Addition of 20 mM bicarbonate did not cause any significant change in PAHs removal rate. A similar result was also reported by Bach et al. (2005). Li et al. (2011) observed that addition of 1,160 mg L^{-1} of Mn(IV) to a PAHs degrading enriched bacterial consortium decreased the degradation rates of fluorene, phenanthrene, fluoranthene, and pyrene by 31–70%. The effect of bio-stimulating agents such as fertilizers, labile carbon sources in the form of organic acids, and surfactants was studied by Agarry and Owabor (2011). When added individually, all the amendments such as Tween 80, silicone oil, pig dung, and NPK fertilizer stimulated the degradation of naphthalene and anthracene in marine sediment. Pig dung and a mixture of pig dung with Tween 80 were the best in PAHs removal when tested individually or in combination. Organic acids such as acetate, lactate, and pyruvate were also found to be stimulatory in PAHs degradation (Chang et al. 2002, 2008; Bach et al. 2005). Langenhoff et al. (1996) reported that naphthalene degradation in a nitrate-reducing sediment column was only commenced after the addition of benzoate.

Very recently, Qin et al. (2018) investigated the effect of Fe^{2+}, Zn^{2+}, Cu(II), Mn^{2+}, Hg^{2+}, Co^{2+}, Pb^{2+}, and Cd^{2+} on BaP degradation by a nitrate-reducing

bacterium, *Cellulosimicrobium cellulans* CWS2. Among the metal ions, Cd^{2+} inhibited the degradation; Fe^{2+}, Zn^{2+}, Hg^{2+}, and Pb^{2+} did not show any toxic effect, while other metals slightly decreased the degradation rate.

14.4 Biosurfactants

Low solubility of PAHs in water is a major degradation rate-limiting factor. Biosurfactants are amphiphilic detergent-like molecules that promote release of sorbed PAHs and increase their aqueous concentrations by emulsification or solubilization process (Deziel et al. 1996; Ron and Rosenberg 2002; Johnsen et al. 2005; Mulligan 2005). The role of biosurfactants in aerobic PAHs degradation was better investigated. However, reports on biosurfactant production and role of biosurfactants in anaerobic degradation of hydrocarbon compounds are rare. Yan et al. (2017) reported biosurfactant production by iron-reducing facultative anaerobic strain, *Hydrogenophaga* sp. PYR1, that could degrade pyrene and BaP. Iron(III) stimulated the biosurfactant production, and the best production was observed with ferric citrate. Biosurfactant production by PAHs-degrading anaerobic bacteria and mechanism of enhanced anaerobic biodegradation by biosurfactants remain unknown.

15 Genetics and Biochemistry of Anaerobic PAHs Biodegradation

Our current understanding of the molecular mechanism of PAHs degradation is limited. Molecular mechanisms of anaerobic degradation of naphthalene and 2-MN in SRB are well studied. Difficulties in maintaining anaerobic cultures and their enzymes and extremely slow growth rate of anaerobic PAHs-degrading bacteria are the two main factors that hinder in-depth molecular investigation of anaerobic PAHs metabolism (Foght 2008; Meckenstock et al. 2016). Most of the information related to anaerobic degradation of naphthalene and 2-MN has been garnered from sulfate-reducing pure culture, NaphS2, and a freshwater enrichment culture, N47 (Meckenstock et al. 2016). The pathways for anaerobic BaP degradation by nitrate-reducing bacteria, *Cellulosimicrobium cellulans* CWS2 (Qin et al. 2018), *Microbacterium* sp. (Qin et al. 2017), and *Pseudomonas* sp. JP1 (Liang et al. 2014), and iron-reducing bacterial strain, *Hydrogenophaga* sp. PYR1 (Yan et al. 2017), have been proposed. Anaerobic degradation pathways of naphthalene and 2-MN in NaphS2 and N47 under sulfate-reducing conditions are analogous to anaerobic benzene and toluene degradation pathways, respectively (Foght 2008; Meckenstock et al. 2016). In contrast, the proposed pathways for anaerobic degradation of HMW PAHs, for example, BaP, in several nitrate- and iron-reducing pure cultures indicate

that they are entirely different from anaerobic naphthalene and 2-MN degradation pathways. This variation could be specific for PAHs (LMW vs HMW) or reducing conditions (facultative anaerobic vs strict anaerobic or nitrate-reducing vs sulfate-reducing conditions) or organism-specific. In view of the currently available information, anaerobic metabolism of naphthalene and 2-MN is summarized in detail, and metabolism of phenanthrene and BaP has been outlined briefly in the following subsections.

15.1 Mechanism of Naphthalene Biodegradation

15.1.1 Initial Activation of Naphthalene

At least three different activation mechanisms, i.e., hydroxylation, carboxylation, and methylation, of naphthalene under entirely anaerobic conditions have been proposed (Fig. 6). Bedessem et al. (1997) detected an unresolved isomer naphthalenol (naphthol) as a principal intermediate during naphthalene degradation under sulfate-reducing conditions. Therefore, hydroxylation was proposed as the initial activation mechanism. Several lines of supporting evidence bake this plausible hypothesis. Indeed, benzene could be hydroxylated to phenol under methanogenic and sulfate- and iron-reducing conditions (Vogel and Grbic-Galic 1986; Caldwell and Suflita 2000). Moreover, phenol formation from benzene has recently been reported in an anaerobic benzene-oxidizing bacterium, *Geobacter metallireducens* (Zhang et al. 2013). Ideally, an activated intermediate of a recalcitrant compound should be readily utilizable by the degrading bacteria. Expectedly, Mihelcic and Luthy (1988a) observed that naphthol was more readily degraded than naphthalene by nitrate-reducing enrichment culture. On the contrary, the inability of several sulfate-reducing and naphthalene-degrading bacteria in using naphthol as substrate and absence of the compound in cellular metabolites exclude it as an intermediate of the metabolic pathways (Zhang and Young 1997; Meckenstock et al. 2000; Musat et al. 2009; Kleemann and Meckenstock 2011).

Most of the available literature dealing with anaerobic naphthalene degradation support carboxylation as the first activation step (Fig. 6). Zhang and Young (1997) reported the accumulation of 2-NA in the supernatant of a naphthalene-degrading and sulfate-reducing enrichment culture. The culture could also use 2-NA for growth without any lag indicating the ready degradability of the compound as a typically activated compound. To confirm the origin of the carboxyl group, the enrichment culture was incubated in the presence of naphthalene and ^{14}C-labeled bicarbonate. Results indicated the incorporation of $^{14}CO_2$ to the most negative carbon atom (C2) of the naphthalene ring.

Interestingly, carboxylation is not exclusive for naphthalene; phenanthrene-carboxylic acid was also detected in phenanthrene-amended sulfate-reducing enrichment culture (Zhang and Young 1997). The position of the carboxylation in phenanthrene ring remained unknown for many years. Using deuterated

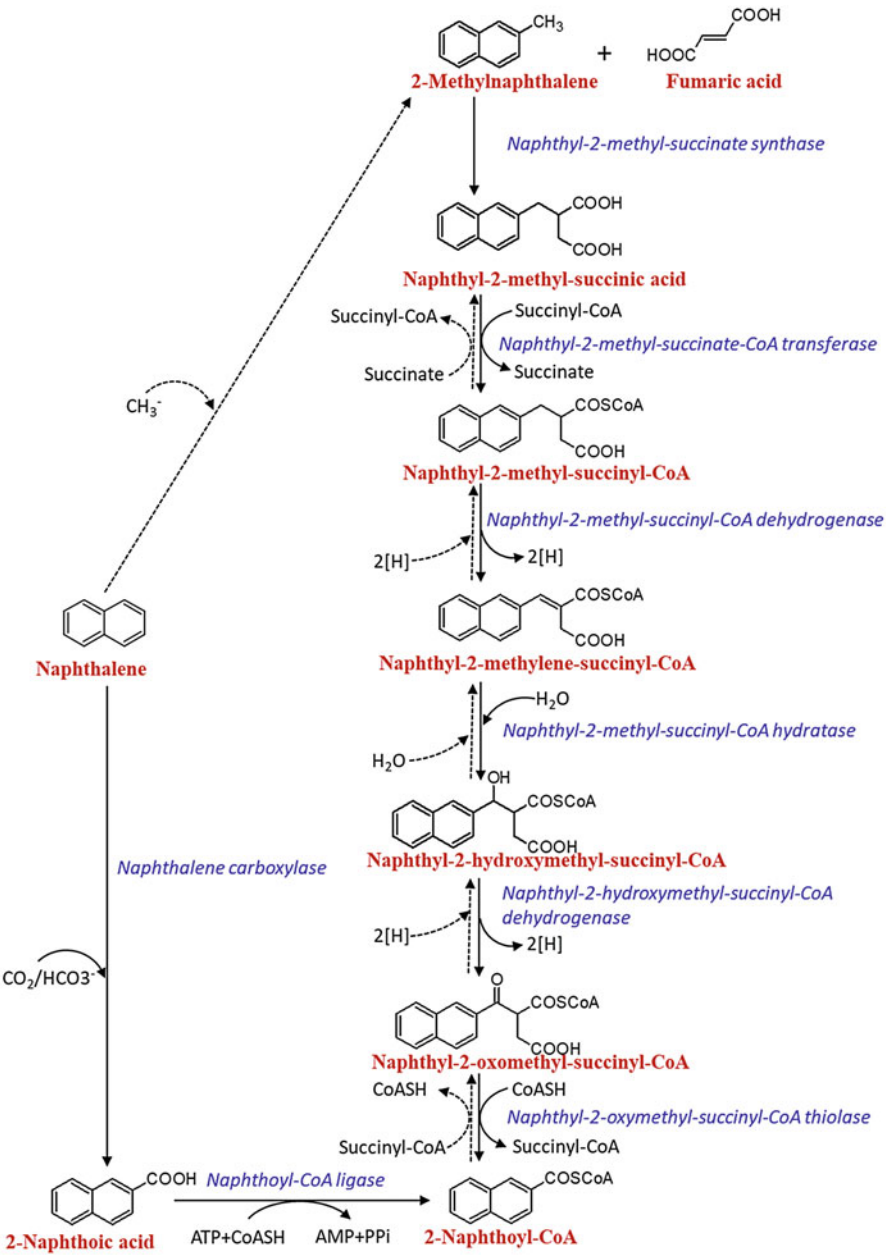

Fig. 6 The reactions of the upper pathway of anaerobic naphthalene and 2-methylnaphthalene degradation. Solid arrows direct to the formation of 2-NA-CoA ester following initial activation of naphthalene by carboxylation reaction and 2-MN activation by fumarate addition reaction. Dotted arrows indicate the alternative pathways

phenanthrene and ^{13}C-labeled bicarbonate, Davidova et al. (2007) unequivocally presented the evidence that phenanthrene is also carboxylated at the C2 position, and the reaction produces 2-phenanthrenecarboxylic acid. Furthermore, carboxylation is not unique to SRB; 2-NA has also been detected as a primary metabolite during naphthalene degradation by iron-reducing bacteria (Kleemann and Meckenstock 2011). Notably, several in situ metabolic profiling investigations identified carboxylated PAHs including 2-NA in PAHs-contaminated sites (Gieg and Toth 2017). Carboxylated PAHs are neither synthesized commercially nor occur as by-products of any known biological reactions; so the presence of these compounds in contaminated sites indicates that carboxylation may be a common activation reaction for many, if not all, PAHs. However, carboxylated intermediates are absent in cellular metabolites of phenanthrene- and BaP-degrading cultures (Tsai et al. 2009; Liang et al. 2014; Qin et al. 2017, 2018; Yan et al. 2017). Likewise, Toth et al. (2018) did not find any carboxylated, methylated, or fumarate substituted naphthalene metabolites in a methanogenic naphthalene-degrading enrichment culture. Therefore, a generalized activating step for all PAHs under anaerobic condition perhaps does not exist.

In sulfate-reducing enrichment culture N47, naphthalene is carboxylated by the enzyme naphthalene carboxylase. The enzyme activity was demonstrated in a crude cell extract of N47 (Mouttaki et al. 2012). In the reaction mixture of a crude cell extract of N47, naphthalene and ^{13}C-bicarbonate, this enzyme produced ^{13}C-labeled 2-NA at a rate of 0.12 nmol min^{-1} mg^{-1} of protein. Interestingly, the enzyme activity was ATP-independent. Divalent cations (Mn^{2+} and Mg^{2+}) and chelating agent (EDTA) do not affect the activity. Moreover, the enzyme is biotin-independent, susceptible to oxygen exposure, and affected by strong reducing agents like sodium dithionite and Ti(III)citrate. Many of the characteristics of the naphthalene carboxylase enzyme suggest that it belongs to UbiD-like carboxylase enzyme family (Meckenstock et al. 2016). A recent proteogenomic study has revealed the presence of an alpha-subunit of the putative naphthalene carboxylase in N47 (Bergmann et al. 2011b). The ORF of the putative carboxylase shows 48 and 45% sequence similarity to alpha-subunit of phenylphosphate carboxylase (PpcA) of *Aromatoleum aromaticum* EbN1 (Rabus et al. 2005) and putative anaerobic benzene carboxylase of the iron-reducing and benzene-degrading culture, BF (Abu Laban et al. 2010), respectively. Also, molecular mass (53.23 kDa) and peptide chain length (481 amino acids) of putative naphthalene carboxylase are almost analogous to those of the EbN1 PpcA (53.99 kDa and 485 amino acids, respectively). Despite the similarities, naphthalene carboxylase is different from PpcA as the latter requires ATP and phosphorylated substrate.

A completely different mechanism of naphthalene activation was also proposed for sulfate-reducing Deltaproteobacterial culture, N47 (Safinowski and Meckenstock 2006). According to the proposal, naphthalene is first methylated to 2-MN and then subsequently transformed to the central metabolite 2-NA (Fig. 6). This pathway is analogous to the anaerobic conversion of benzene to toluene (Coates et al. 2002). The methylation theory is based on the detection of naphthyl-2-methylsuccinic acid (NMS) and naphthyl-2-methylenesuccinic acid (NeMS) in

naphthalene-supplemented N47 culture. These two metabolites are highly specific for anaerobic 2-MN degradation; Safinowski and Meckenstock (2006) therefore reasoned that the metabolites were produced from 2-MN which, in turn, was derived from naphthalene by a methylation reaction.

Additionally, when naphthalene-degrading N47 culture was transferred to 2-MN-amended medium, rapid onset of degradation occurred without a pronounced lag (Safinowski and Meckenstock 2006). However, in vice versa scenario, i.e., when 2-MN-adapted culture was transferred to naphthalene-amended medium, degradation commenced after a long period of ~100 days. The authors suggested that naphthalene-grown cells contained all necessary enzyme(s) required for 2-MN degradation, while the enzyme(s) were not induced in 2-MN-grown cells that caused such a long adaptation period. While it came to the origin of the methyl group, the authors argued that it could be generated from bicarbonate via a reverse carbon monoxide (CO) dehydrogenase pathway. CO dehydrogenase activity of about 0.0974 μmol min^{-1} mg^{-1} of protein was found in culture N47 using methyl viologen as the TEA. Although methylation is not regarded as a primary mechanism of benzene activation during anaerobic degradation, Ulrich et al. (2005) detected toluene in benzene-degrading and nitrate-reducing enrichment culture. Like benzene, naphthalene could also be methylated, as it is more reactive than benzene in electrophilic substitution and addition reaction. According to quantum mechanical calculations, the net loss in stabilization energy for the first step in electrophilic substitution or addition is higher for benzene than that for naphthalene. Therefore, it is expected that electrophilic substitution will occur for naphthalene if it reacts with strong methyl group-donating compounds. However, later studies on N47 failed to detect methylated derivative in culture incubated with naphthalene and a methyl group donor such as methyltetrahydrofolate, *S*-adenosyl-L-methionine, and methylcobalamin (Mouttaki et al. 2012).

Convincing evidence that cancels out methylation as an initial activation mechanism has been provided by Musat et al. (2009). When naphthalene-grown cells of three sulfate-reducing pure bacterial strains, NaphS2, NaphS3, and NaphS6, were transferred to 2-MN, substrate utilization did not commence before a long adaptation period. The second evidence was obtained by growing NaphS2 in deuterated(d_8) naphthalene and unlabeled 2-MN. Most of the d_8 label was found in the carboxylation product 2-NA, while succinic acid adduct 2-naphthylmethylsuccinate was found unlabeled. In addition, gel electrophoresis of 2-MN-grown cell extracts revealed high-molecular-mass co-migrating protein bands. These protein bands were only specific for 2-MN-grown cells, indicating that the bacteria use different activation mechanisms for naphthalene and 2-MN. Nevertheless, traces of 2-MN-specific metabolites were also detected in naphthalene-grown cultures of NaphS2, NaphS3, and NaphS6 (Musat et al. 2009). The authors argued that these metabolites were probably produced via back reaction starting from 2-naphthoyl-CoA and succinyl-CoA. Recently, enzymes specific for 2-MN degradation have been found in several naphthalene-degrading and sulfate-reducing cultures of *Desulfobacteraceae* (Kummel et al. 2015). One possible explanation for this scenario may be the co-induction of 2-MN-specific gene clusters by naphthalene.

15.1.2 Initial Activation of 2-Methylnaphthalene

In many senses, anaerobic degradation 2-MN and toluene show striking similarities. Most of the information on anaerobic toluene degradation has been obtained from *Azoarcus* sp. strain T (Beller and Spormann 1997) and *Thauera aromatica* (Biegert et al. 1996). Anaerobic toluene degradation in these organisms initiates with the conversion of toluene to benzylsuccinic acid by the enzyme benzylsuccinate synthase (Bss) that catalyzes the addition of fumarate to the methyl side chain of toluene. Likewise, the initial activation of 2-MN involves similar fumarate addition step that leads to the production of naphthyl-2-methylsuccinic acid by the catalytic action of 2-naphthylmethylsuccinate synthase (Nms) (Fig. 6). The enzyme reaction was demonstrated in a dense cell suspension of culture N47 with fumarate (Safinowski and Meckenstock 2004). Musat et al. (2009) observed 2-MN-specific co-migrating protein band in gel electrophoresis of cell extract from sulfate-reducing cultures, NaphS2, NaphS3, and NaphS6. Peptide sequencing of the putative large subunit of Nms (NmsA) revealed that the protein shares sequence similarity to the large catalytic subunit of Bss (BssA). Whole genome and proteome sequencing of the culture N47 revealed Nms genes, *nmsA*, *nmsB*, and *nmsC*, corresponding to the three subunits of the Nms protein. The α (95.9 kDa), β (7.9 kDa), and γ (7.8 kDa) subunits share sequence similarities to the corresponding subunits of Bss enzyme. Notably, the NmsA sequences of N47 show more similarity (92%) to those of NaphS6 compared to any BssA sequences. In addition to the gene products of *nmsABC*, NmsD, a putative Nms-activating enzyme that shares sequence similarity to putative 1-methyl alkyl-succinate synthase activase (MasG) of *Azoarcus* sp. strain HxN1, was also identified in N47 (Selesi et al. 2010).

During 2-MN degradation by a sulfate-reducing consortium that was enriched from marine harbor estuarine sediment, Sullivan et al. (2001) found very different degradation metabolites. They argued that 2-NA is not a product of direct carboxylation or fumarate addition. Instead 2-NA was proposed to be generated via the oxidation of the methyl side chain. Also, several carboxylated-2-MN metabolites and their reduced derivatives were detected. As in the case of naphthalene, several alternative activation mechanisms of 2-MN activation, i.e., oxidation and carboxylation, may exist in nature.

15.1.3 Conversion of Naphthyl-2-Methylsuccinic Acid to 2-NA

Despite the difference in naphthalene and 2-MN activation mechanisms, both the substrates channeled to the central metabolic pathway via the common intermediate 2-NA. Unlike one-step naphthalene activation reaction (naphthalene to 2-NA), conversion of 2-MN to 2-NA via naphthyl-2-methylsuccinic acid (NMS) is a multistep process (Fig. 6). As in toluene degradation, the reactions following NMS generation proceed via the formation of corresponding CoA ester, NMS-CoA. In a subsequent reaction, NMS-CoA is oxidized to naphthyl-2-methylenesuccinyl-CoA

(NMeS-CoA) by naphthyl-2-methylsuccinyl-CoA dehydrogenase activity. The activity of the succinyl-CoA:naphthyl-2-methylsuccinate CoA-transferase enzyme, which converts NMS to the corresponding CoA ester, has been measured in crude cell extract of culture N47. This CoA-transferase enzyme shares significant similarity to succinyl-CoA:(R)-benzylsuccinate CoA-transferase, and both belong to the family(III) of CoA-transferases. In addition, naphthyl-2-methylsuccinyl-CoA dehydrogenase activity has also been measured in crude extract of N47 (Safinowski and Meckenstock 2004). The rest of the steps leads to the conversion of NMeS-CoA to 2-NA. The reaction series should proceed via the formation of oxidized intermediates as in benzoyl-CoA pathway of anaerobic toluene degradation. However, the predicted intermediates, naphthyl-2-hydroxymethyl-succinyl-CoA and naphthyl-2-oxomethyl-succinyl-CoA, are not yet detected in any 2-MN-degrading culture extracts. Nevertheless, recent proteogenomic studies on culture N47 (Selesi et al. 2010; Bergmann et al. 2011a) and NaphS2 (Didonato et al. 2010) provide interesting clues suggesting the presence of all the necessary genes and enzymes for the putative oxidation reactions. The *bns* (beta-oxidation of naphthyl-2-methylsuccinate) operon in N47 consists of eight genes, *bnsABCDEFGH*. Functions of the gene products were predicted from orthologous sequences of toluene-degrading *Aromatoleum aromaticum* EbN1, *Thauera aromatica*, and *Azoarcus* sp. strain T (Meckenstock et al. 2016).

15.1.4 Formation of 2-Naphthoyl-CoA from 2-NA by CoA Ligase

Anaerobic metabolism of aromatic acid proceeds via the formation of a thioester with coenzyme A (CoA) by CoA ligase or CoA-transferase enzyme (Fuchs et al. 2011). Observing the formation of 2-NA and reduced 2-NA derivatives, Zhang et al. (2000) speculated that 2-NA might be thioesterified with CoA-SH so that the subsequent reactions would proceed through the formation of ring reduction products in a manner that is similar to the anaerobic benzoyl-CoA pathway (Harwood et al. 1998). Indeed, the –CO-S-CoA group facilitates further electron transfer and ring reduction by lowering the midpoint potential of the first electron transfer process (Johann and Georg 1997). A phototrophic bacterium, *Rhodopseudomonas palustris*, produces a 4-hydroxybenzoate-coenzyme A ligase that can catalyze the MgATP-requiring thioesterification reaction with 4-hydroxybenzoate (or benzoate) and coenzyme A. Benzoate-CoA ligase of a facultative anaerobe, *T. aromatica*, can also perform benzoyl-CoA-forming catalysis in the presence of benzoate, MgATP, and coenzyme A (Schühle et al. 2003). An indication for the presence of similar CoA ligase enzyme in NaphS2 and N47 has been obtained from proteogenomic investigations (Didonato et al. 2010; Bergmann et al. 2011b). *Deltaproteobacterium* NaphS2 genome contains a gene (*NPH_5477*) that encodes a putative 2-NA-CoA ligase with 50% similarity to *E. coli* phenylacetate-CoA ligase. During differential growth on benzene and naphthalene, *NPH_5477* was upregulated (Didonato et al. 2010). Sequence comparison between 2-NA-CoA ligase in N47 and benzoate-CoA ligase of *R. palustris* revealed the presence of several ORFs. Among the most related

ORFs, *N47_B20660* was found to be expressed exclusively in naphthalene- and 2-NA-grown cultures (Bergmann et al. 2011b). However, experimental evidence confirming the presence and activity of 2-NA-CoA ligase has not yet been provided.

15.1.5 Ring Reduction of 2-NA

Following the formation of common 2-NA intermediate, both naphthalene and 2-MN degradation proceeds through identical sequential ring reduction steps that ultimately pave the way of ring cleavage (Fig. 7). Early GC-MS analysis of culture extract from the culture N47 provided clues for the formation of several metabolites by ring reduction (Meckenstock et al. 2000). Two tetrahydro-derivatives, 1,2,3,4-tetrahydro-2-naphthoic acid (1,2,3,4-THNA) and 5,6,7,8-tetrahydro-2-naphthoic acid (5,6,7,8-THNA), in addition to octahydro-2-NA and decahydro-2-NA, in naphthalene-fed culture were detected (Meckenstock et al. 2000). A similar analysis on another naphthalene-degrading and sulfate-reducing enrichment culture extracts also revealed the formation of five-ring reduction products: dihydro-; 5,6,7,8-tetrahydro-; hexahydro-; octahydro-; and decahydro-2-NA (Zhang et al. 2000). The formation of these metabolites has suggested that 2-NA is sequentially reduced through five successive steps toward the production of decahydro-2-NA. However, decahydro-2-NA was proposed as a dead-end metabolite (Annweiler et al. 2002).

Detection of only deuterated 5,6,7,8-THNA (but not 1,2,3,4-THNA) in the study by Zhang et al. (2000) has provided an important clue that the ring reduction might start in the non-substituted ring (ring II) rather than the carboxyl-substituted ring (ring I). On the other hand, accumulation of 1,2,3,4-THNA, even in small amounts, together with 5,6,7,8-THNA in the study of Meckenstock et al. (2000) could not deduce where the first reduction was initiated from the metabolites' profile. If the ring reduction starts at the ring I, then 1,2,3,4-THNA should have been the major metabolite. To resolve the apparent discrepancy, Annweiler et al. (2002) analyzed the metabolites formed while growing the culture N47 on 1,2,3,4-tetrahydronaphthalene (tetralin). Only 5,6,7,8-THNA was detected in culture extract indicating that the addition of C1 unit requires an aromatic ring and the ring reduction starts from the unsubstituted ring II. However, recent metabolite profiling studies have reported the presence of 1,2,3,4-THNA in PAHs-contaminated samples (Aitken et al. 2004; Griebler et al. 2004; Wawrik et al. 2012). Therefore, ring reduction may initiate in either of the rings. Conditions that favor a reduction process are still unknown.

High resonance energy of mono- or polyaromatic compounds makes a ring cleavage reaction very challenging in a biological system. Before cleavage of the ring structure, anaerobic bacteria adopt a strategy that involves the dearomatization of the ring. Reductive dearomatization of the central metabolite benzoyl-CoA is a well-known process in anaerobic degradation of monoaromatic BTEX compounds (Boll 2005; Fuchs et al. 2011). The dearomatization process that leads to the production of 1,5-dienoyl-CoA (cyclohex-1,5-diene-1-carboxy-CoA) is catalyzed by benzoyl-CoA reductase (Bcr) enzyme (Boll and Fuchs 1995; Fuchs et al. 2011).

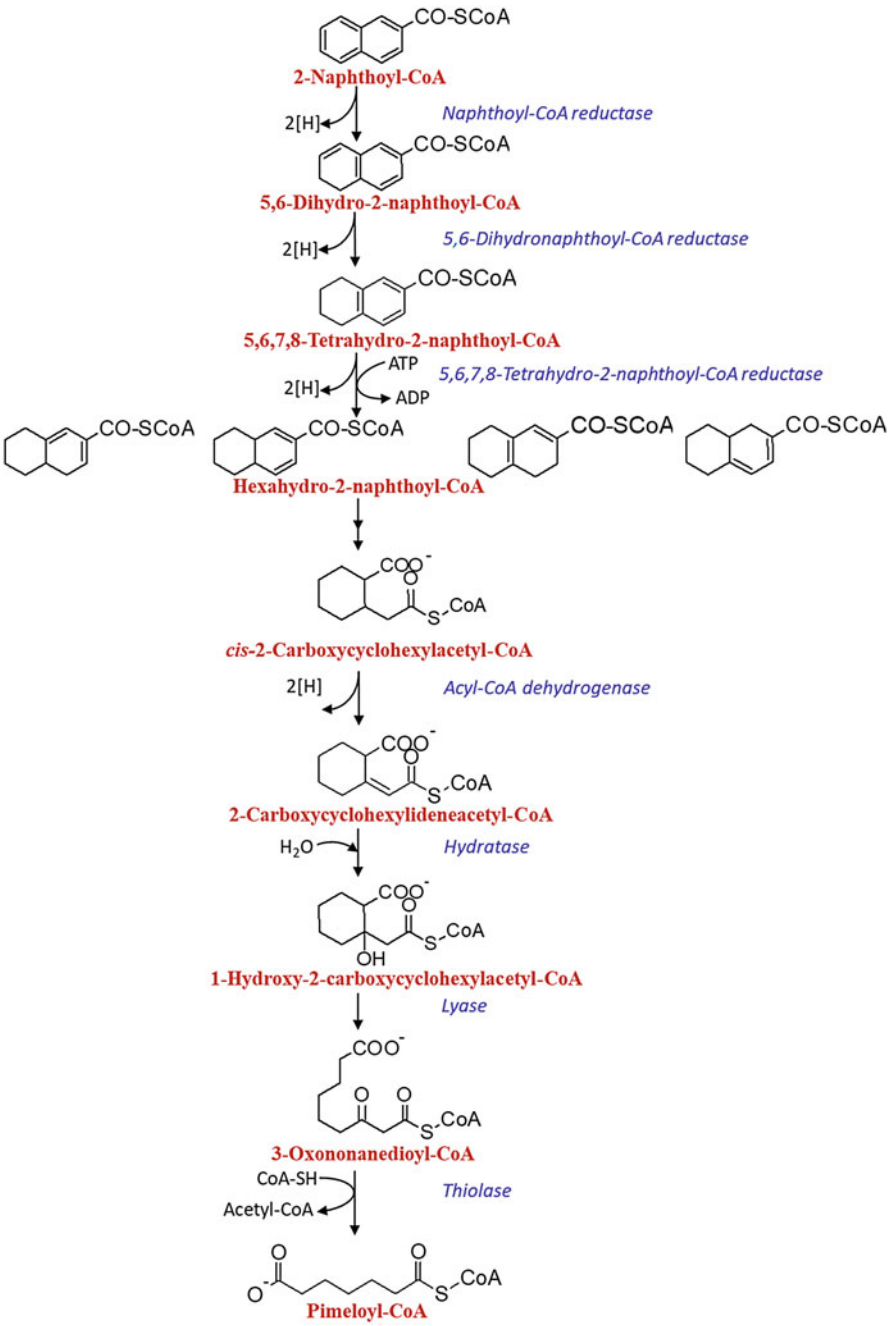

Fig. 7 Formation of the lower pathway central metabolite pimeloyl-CoA from 2-naphthoyl-CoA via sequential ring reduction and two ring-opening reactions in the anaerobic degradation of naphthalene and 2-MN

Two classes of Bcr have been reported so far. They catalyze the formation of the same product but differ in some properties; ATP dependence is one of the most striking features of the enzyme. Bcr I activity has been reported so far in *Thauera aromatica* (Breese et al. 1998), *Rhodopseudomonas palustris* (Egland et al. 1997), and *Azoarcus evansii* (Harwood et al. 1998). Oxygen-sensitive Bcr I catalyzes two-electron transfer from reduced ferredoxin to the substrate with stoichiometric hydrolysis of two molecules of ATP to ADP and PPi. On the other hand, class II Bcr activity in obligate anaerobic *Geobacter metallireducens* does not require ATP (Kung et al. 2009). N47 genome contains genes that are similar to the genes encoding the four subunits of class I Bcr in *Azoarcus* sp. However, the gene similar to *BamB* that codes for the active site of Bcr II is absent in N47. In contrast, NaphS2 genome harbors gene analogous to both classes of Bcr (Didonato et al. 2010). The activity of the putative naphthoyl-CoA reductase (Ncr) has been shown in the crude cell extracts of N47 which catalyzes sodium dithionite-dependent four-electron transfer reaction, converting NCoA to 5,6,7,8-THN-CoA. Although sequences of *ncrABCD* and *bcrABCD* are similar, unlike Bcr I protein, Ncr activity in N47 is independent of ATP and insensitive to oxygen indicating the novelty of Ncr within the family of reductases (Eberlein et al. 2013b). Ncr has been purified and characterized from N47 culture. The enzyme is a 150 kDa dimeric protein consisting of two 72 kDa subunits. It contains FMN and FAD cofactors and [4Fe-4S] clusters. It is classified as a member of old yellow enzyme (OYE) family based on the presence of flavin cofactors and iron-sulfur cluster as well as sequence similarity to cyclohexa-1,5-diene-1-carboxyl-CoA oxidase from *T. aromatica* (Eberlein et al. 2013a).

Notably, no dihydro derivative formation was observed during NCoA reduction. However, when *ncr* gene of N47 (*N47_G38220*) was heterologously expressed in *Escherichia coli*, the extract of recombinant cells catalyzed the conversion of NCoA to dihydro-2-naphthoyl-CoA (DHN-CoA) rather than 5,6,7,8-THN-CoA (Eberlein et al. 2013a). Further, Estelmann et al. (2015) investigated the expression of three putative *ncr* (*N47_G38220* from N47 and *NPH_5475* and *NPH_1753* from NaphS2) in *E. coli* host. All *ncr* gene products could convert NCoA to a two-electron reduced metabolite 5,6-dihydro-2-naphthoyl-CoA (5,6-DHN-CoA). However, none of the enzymes showed a four-electron reduction of NCoA to THN-COA. Genes encoding putative 5,6-DHN-CoA reductases from both N47 (*N47-G38210*) and NaphS2 (*NPH_5476*) were expressed in *E. coli*. The gene products catalyzed the formation of 5,6,7,8-THN-CoA from 5,6-DHN-CoA (Estelmann et al. 2015). Subsequent reduction of 5,6,7,8-THN-CoA to hexahydro-2-naphthoyl-CoA (HHNCoA) in N47 involves another two-electron reduction step that is catalyzed by an ATP-dependent and oxygen-sensitive THN-CoA reductase enzyme (Eberlein et al. 2013b). Therefore, reduction of NCoA to HHNCoA involves three enzymatic reduction steps. The first two enzymes in this series belong to the OYE family, and the other, HHNCoA, is identical to class I Bcr family. In both N47 and NaphS2, genes encoding four putative subunits of THN-CoA reductase have been identified as a part of a gene cluster. In N47, the gene cluster forms *thn* operon. Protein prediction from the operon indicates that the operon contains genes that may encode several putative enzymes in addition to THN-CoA reductase. The predicted enzymes

include an oxidoreductase, enoyl-CoA hydratases/hydrolases, acyl-CoA dehydrogenases, 3-hydroxyacyl-CoA dehydrogenases, and acetyl-CoA thiolases/transferases (Meckenstock et al. 2016). By comparing the putative functions of the enzymes with the functions of analogous enzymes in benzoyl-CoA pathway, Meckenstock et al. (2016) hypothesized that downstream degradation of hexahydronaphthoyl-CoA could proceed via β-oxidation-like reactions, and the first ring cleavage would occur through a thiolytic cleavage of acetyl-CoA producing a cyclohexanoic acid-CoA ester derivative.

15.1.6 Ring Cleavage of Reduced 2-NA Products

The downstream ring cleavage pathways in anaerobic naphthalene and 2-MN degradation proceed through cyclohexanoic acid rather than monoaromatic compound (Annweiler et al. 2002; Weyrauch et al. 2017). In naphthalene-degrading N47 culture extracts, GC-MS analysis revealed the presence of two ring cleavage products (Annweiler et al. 2002). The first product consisted of a cyclohexane ring with two carboxylic acid side chains with $C_{11}H_{16}O_4$-diacid. The exact constituents of the diacid side chains are not known yet. The second ring cleavage product was detected as a *cis*-2-carboxycyclohexylacetic acid that was assumed to be a β-oxidation product of the diacid. Recently, metabolism of *cis*-2-carboxycyclohexylacetic acid-CoA ester (2-(carboxymethyl)cyclohexane-1-carboxylic acid-CoA ester) by N47 culture extracts has been demonstrated by Weyrauch et al. (2017). When *cis*-2-carboxycyclohexylacetic acid-CoA ester (m/z = 936) was incubated with a cell-free extract of the culture N47 and NaphS2 in the presence of ferrocenium hexafluorophosphate as the artificial electron acceptor, two new compounds were detected in GC-MS representing m/z values of 934 and 952, respectively. This indicates that metabolism of *cis*-2-carboxycyclohexylacetic acid proceeds via α-, β-desaturation and a subsequent water addition at the β-position. The dehydrogenase and hydratase enzymes catalyzing the two reactions show similarity in reaction to corresponding enzymes of branched acyl-CoA ester metabolism. The acyl-CoA dehydrogenase introduces a double bond to *cis*-2-carboxycyclohexylacetic acid producing 2-carboxycyclohexylideneacetyl-CoA that is converted into 1-hydroxy-2-carboxycyclohexylacetyl CoA in the hydratase-catalyzed reaction. The remaining cyclohexane ring opening and central acetyl-CoA metabolism substrates production proceed via pimeloyl-CoA. Before converting to CoA ester of dicarboxylic C7 pimelic acid, the ring structure of 1-hydroxy-2-carboxycyclohexylacetic acid-CoA ester is opened by a novel class of ring-cleaving lyase. In the subsequent thiolytic cleavage reaction, the ring cleavage reaction product, 3-oxononanedioyl-CoA, is converted to pimeloyl-CoA by thiolase (Weyrauch et al. 2017).

Further degradation of pimeloyl-CoA to glutaryl-CoA proceeds via β-oxidation (Fig. 8). Pimeloyl-CoA is converted sequentially to 2,3-dehydropimeloyl-CoA, 3-hydroxypimeloyl-CoA, and 3-oxopimeloyl-CoA probably by acyl-CoA dehydrogenase, acyl-CoA hydratase, and β-hydroxyacyl-CoA dehydrogenase, respectively. Although no peak representing glutaryl-CoA was detected during pimeloyl-CoA conversion, cell-free extracts of both N47 and NaphS2 strains can utilize glutaryl-

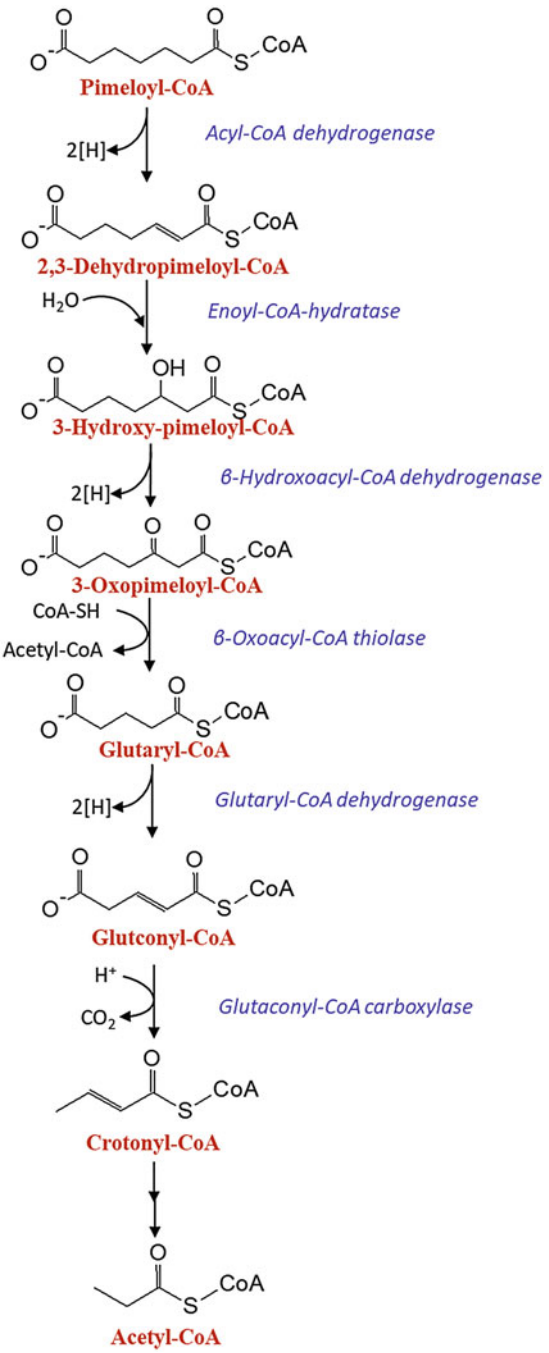

Fig. 8 Lower pathway of anaerobic naphthalene and 2-methylnaphthalene degradation. Pimeloyl-CoA is converted to the TCA cycle intermediate acetyl-CoA via glutaryl-CoA. Pimeloyl-CoA is derived from 2-naphthoyl-CoA that is in turn generated during the anaerobic oxidation of the parent substrates

CoA (Weyrauch et al. 2017). In the glutaryl-CoA assay, four major peaks appeared in LC-MS chromatogram representing glutaconyl-CoA, crotonyl-CoA, 3-hydroxybutyryl-CoA, and acetyl-CoA. A similar conversion process of glutaryl-CoA to glutaconyl-CoA and glutaconyl-CoA to crotonyl-CoA has been previously reported in strict anaerobes (Schöcke and Schink 1999; Müller and Schink 2000; Wischgoll et al. 2009). Unlike facultative anaerobes that exploit decarboxylating glutaryl-CoA dehydrogenase only for the direct conversion of glutaryl-CoA to crotonyl-CoA, strict anaerobes use non-decarboxylating glutaryl-CoA dehydrogenase and glutaconyl-CoA decarboxylase to produce crotonyl-CoA from glutaryl-CoA via glutaconyl-CoA. By the formation of TCA cycle intermediate, acetyl-CoA, the pathway merges to the central respiration pathway.

15.2 Mechanism of Phenanthrene and BaP Biodegradation

As mentioned above, phenanthrene degradation in a sulfate-reducing enrichment culture starts with the carboxylation at the C2 position (Davidova et al. 2007). Recently, Himmelberg et al. (2018) detected 2-phenanthroic acid as the primary metabolite in phenanthrene-degrading and sulfate-reducing enrichment culture. Detection of carboxylated phenanthrene also suggests that the anaerobic phenanthrene metabolism might follow analogous steps as in naphthalene biodegradation. The study of Himmelberg et al. (2018) also provided some clues that indicate possibility for the existence of a similar metabolic route. Like 2-NA, 2-phenanthroic acid is also converted to corresponding CoA ester by the enzyme 2-phenanthroate-CoA ligase. Moreover, several ring-reduced products were also identified, indicating the occurrence of ring reduction steps that will make the ring cleavage possible at later stages. Tsai et al. (2009) detected phenol and *p*-cresol in phenanthrene-degrading and sulfate-reducing enrichment culture. The authors proposed that phenanthrene degradation proceed through a series of hydration and hydrolysis reactions and a decarboxylation reaction on *p*-cresol. Phenol was also detected in the fluorene-amended cultures.

Reports proposing anaerobic BaP degradation mechanism are extremely sporadic. BaP degradation pathways have been reported for facultative anaerobes, *Pseudomonas* sp. JP1 (Liang et al. 2014), *Microbacterium* sp. (Qin et al. 2017), and *Cellulosimicrobium cellulans* CWS2 (Qin et al. 2018), and biosurfactant-producing and iron-reducing *Hydrogenophaga* sp. PYR1 (Yan et al. 2017). The metabolites of BaP degradation identified so far are listed in Table 7. If BaP biodegradation is thought to proceed via a pathway that is comparable to naphthalene degradation pathway, carboxylated BaP should be a metabolite in upper degradation pathway. However, such a metabolite is not reported yet in any BaP-degrading culture. Available proposed BaP degradation pathway does not merge at a central metabolite like benzoic acid or naphthoic acid as in benzene and naphthalene degradation, respectively. In addition, a common activation reaction in BaP-degrading pathway cannot be expected, not only for the absence of carboxylated metabolites, but proposed pathways indicate several distinct first step metabolites even for

Table 7 Metabolites of BaP degradation identified so far in nitrate- and iron-reducing bacteria

Bacterium	TEA	Identified metabolites	Detection system	Reference
Microbacterium sp.	Nitrate	4,5-Dihydrobenzo(a)pyrene; chrysene; 7,8,9,10-tetrahydrochrysene; phenanthrene; 3-acetylphenanthrene; 2-methyl-1-naphthaleneacetic acid; naphthalene; 1-naphthalenol; 5,8-nihydro-1-naphthalenol; 3,8-nihydroxy-3,4-dihydronaphthalen-1(2H)-one; 2-(1-hydroxyethyl) hydroxymethylbenzene; 7,8,9,10-tetrahydrobenzo(a)pyrene; pyrene; 4,5-dihydropyrene; 4,5-dimethylphenanthrene; 4-methylphenanthrene	GC-MS	Qin et al. (2017)
Cellulosimicrobium cellulans CWS2	Nitrate	Pyrene; 1-aminopyrene; phenanthrene; 1-methylphenanthrene; 1,7-dimethylnaphthalene; 1-(2-hydroxypropyl)-naphthalene; 1-hydroxy-3-(3-methyl-2-butenyl)-1,4-naphthalenedione; 1-methylnaphthalene; diethyl phthalate; 2-acetyl-3-methoxybenzoic acid	GC-MS	Qin et al. (2018)
Pseudomonas sp. JP1	Nitrate	1,12-Dimethylbenz(a)anthracene; 7,8,9,10-tetrahydrobenzo(a)pyrene; 5-ethylchrysene; benz(a)anthracene; pyrene; chrysene; 4,5-dimethylphenanthrene; 4-methylphenanthrene; phenanthrene; 1,2,3,4-tetrahydro-4-methyl-4-phenanthrenol; 1-ethyl-2-methylphenanthrene; 1-methylphenanthrene; 2-methylphenanthrene; alpha-methylstilbene; benz(a)anthracene; 2,3-dimethylphenanthrene; 2-methylphenanthrene; 2-methylanthracene; 1-methylanthracene; anthracene	GC-MS	Liang et al. (2014)
Hydrogenophaga sp. PYR1	Iron(III)	5-Ethylchrysene; pyrene; 1H-phenalen-1-one; phenanthrene; benzoic acid-2-hydroxy-phenyl ester; 1,2,3-trimethyl-4-propenyl naphthalene	GC-MS	Yan et al. (2017)

the same strain. For instance, both 4,5-dihydrobenzo(a)pyrene and 7,8,9,10-tetrahydrobenzo(a)pyrene have been suggested as initial reduction step products for BaP-degrading and nitrate-reducing *Microbacterium* sp. (Qin et al. 2017). Similarly, Liang et al. (2014) suggested 1,12-dimethylbenz(a)anthracene, 7,8,9,10-

tetrahydrobenzo(a)pyrene, and 5-ethylchrysene as products of alternative initial reactions. According to Qin et al. (2017), BaP degradation proceeds through initial reduction followed by formation of unsubstituted BaP congeners (pyrene and chrysene) and subsequently through ring opening that leads to the production of naphthalene and its hydroxyl derivatives. In contrast, the pathway proposed by Liang et al. (2014) involves methylated derivatives in addition to the reduced and congener derivatives.

At this moment, reaching a consensus about BaP biodegradation seems difficult. However, proposed pathways indicate the formation of some common metabolites during BaP degradation. Pyrene and phenanthrene have been detected as BaP metabolites in cultures of *Pseudomonas* sp. JP1, *Microbacterium* sp., *Hydrogenophaga* sp. PYR1, and *Cellulosimicrobium cellulans* CWS2 (Qin et al. 2017). Chrysene is the common BaP metabolite in *Pseudomonas* sp. JP1 and *Microbacterium* sp. The same 7,8,9,10-tetrahydrobenzo(a)pyrene has been detected in both *Microbacterium* sp. and *Pseudomonas* sp. JP1. Detection of similar compounds may be indicative of the existence of novel pathway(s) for BaP biodegradation. In contrast, detection of reduced derivatives, 4,5-dihydrobenzo(a)pyrene and 7,8,9,10-tetrahydrobenzo(a) pyrene, indicates the involvement of ring reduction steps as in naphthalene and 2-MN degradation.

16 Metabolite Profiling for Monitoring In Situ Anaerobic PAHs Biodegradation

Degradation metabolites of various environmental contaminants are used as diagnostic biomarkers for investigating the biotic fate of the compounds in the real environments (Callaghan 2013; Gieg and Toth 2017). Metabolite profiling or metabolome analysis provides unambiguous evidence that biodegradation is occurring or has already occurred since metabolites are the products of enzyme-catalyzed reactions. Suitability of a compound as a biomarker is determined by some essential criteria. The metabolite generated during the active biodegradation process must be specific to the parent compound monitored and is not a natural as well as xenobiotic compound and is biodegradable (Callaghan 2013). The selection criteria necessitate in-depth knowledge of the degradation pathway(s) and analytical competence. Availability and continuous improvement of efficient extraction methods and high precision hyphenated separation and detection techniques have made the task of detection of compound-specific biomarker(s) easier for both field and laboratory investigations. However, our current understanding of anaerobic PAHs metabolism is very limited. Insight into the naphthalene and 2-MN metabolism provided some specific metabolites that are used as biomarkers. These include naphthyl-2-methylsuccinic acid, naphthyl-2-methylenesuccinic acid, 2-NA, 5,6,7,8-tetrahydronaphthoic acid, and hexahydronaphthoic acids. Therefore, these metabolites have been used as metabolic markers for monitoring PAHs biodegradation mainly in groundwater samples obtained from PAHs-impacted sites (Table 8).

Table 8 Metabolites of PAHs detected in contaminated environments

Sample type	Site characteristic	Pollutants	Signature compounds	Detection system	Reference
Production water from monitoring well	Coal mine	Coal-derived aliphatic and aromatic compounds	1-Naphthoic acid (1-NA) or 2-NA; methylnaphthoic acid (MNA); dimethylnaphthoic acid (DMNA); 1,2,3,4-tetrahydro-2-naphthoic acid (1,2,3,4-THNA); 5,6,7,8-THNA	GC-MS	Wawrik et al. (2012)
Groundwater from monitoring well	Gasoline impacted aquifer	Gasoline components including naphthalene	2-NA; THNA (isomer unresolved); hexahydro-2-naphthoic acid (HHNA); methylnaphthoic acid (MNA)	GC-MS	Phelps et al. (2002)
Groundwater	Former oil refinery	Fuel gas, liquid propanegas, gasoline, fluid cracking unit coke, heavy fuel oil, kerosene and distillates, asphalt, and others	1- or 2-NA; 5,6,7,8-THNA; MNA; DMNA; 1,2,3,4-THNA; 5,6,7,8-THNA	GC-MS	Aitken et al. (2004)
Oil	Petroleum reservoirs	Oil	2-NA; THNA; two isomers of decahydro-2-naphthoic acid	GC-MS	Parisi et al. (2009)
Groundwater sample monitoring well	Historically contaminated MGP	Hydrocarbon mixture	2-NA; 5,6,7,8-THNA; HHNA; carboxylated-2-naphthoic acid	GC-MS	Oka et al. (2011)
Groundwater sample	Former gas-works site	Tar oil	Naphthyl-2-methylsuccinate (NMS); naphthyl-2-methylene-succinate (NeMS); methylnaphthyl-2-methylsuccinic acid; 2-NA; MNA; acenaphthene-5-carboxylic acid	LC-ESI-MS-MS, Q-TOF MS	Jobelius et al. (2010)
Groundwater sample	Former gas plant facility	Coal and tar-derived compounds	1-NA; 2-NA; 1-naphthylacetic acid; 2-naphthylacetic acid; 1-hydroxy-2-naphthoic acid; 2-hydroxy-3-naphthoic acid; NeMS	LC-ESI-MS-MS	Ohlenbusch et al. (2002)
Groundwater sample monitoring well	Former gas-works site	Tar oil-derived contaminants including BTEX and PAHs	Indanoic acid; MNA; acenaphthenoic acid; acenaphthylenoic acid	GC-MS	Safinowski et al. (2006)
Groundwater sample monitoring well	Former gas-works site	Tar oil-derived contaminants including BTEX and PAHs	1-NA; 2-NA; 1,2,3,4-THNA; 5,6,7,8-THNA; NMS	GC-MS	Griebler et al. (2004)

In addition to the detection of signature metabolites that provide evidence of ongoing or already occurred biodegradation, metabolite profiling provides a strong indication of the presence of yet to be discovered metabolites and even the existence of alternative novel degradation pathway(s). For example, methylnaphthoic acid, which might be a metabolite of either naphthalene or 2-MN or both, has been identified (Aitken et al. 2004). This metabolite is not reported in any PAHs-degrading enrichment or pure culture study. The same is true for 2,6-dimethylnapthoic acid. Detection of indanoic acid, acenaphthenoic acid, and acenaphthylenoic acid indicates that these metabolites result from carboxylation of the parent compounds. Drawing analogy to naphthalene and phenanthrene activation pathways, it can be inferred that the same carboxylating activation mechanism also works for other unsubstituted PAHs. Detection of 1-NA, 2-NA, 1-hydroxy-2-naphthoic acid, and 2-hydroxy-3-naphthoic acid is suggestive of the existence of yet undiscovered degradation pathways. To harness the maximum from a metabolite profiling, further studies in anaerobic degradation of HMW as well as carcinogenic PAHs such as BaP are required.

17 Anaerobic Bioremediation of PAHs

Integrated remediation approaches that combine physical, chemical, and biological treatment technologies have been shown to be more effective than a single treatment (Kuppusamy et al. 2017). However, remediation of anaerobic environments contaminated with PAHs is quite challenging. Anaerobic remediation options available are less compared to aerobic treatment processes and limited in terms of feasibility and applicability. Contamination of anaerobic subsurface soils, aquifers, sediments, and sludge with PAHs is a worldwide problem. Addition of oxygen to anoxic environments for stimulating in situ degradation of organic pollutants has very limited success, and the process encounters technical difficulties (Thomas and Ward 1989; Morgan and Watkinson 1992; Lovley et al. 1994). Several physical and chemical remediation technologies for the removal of the pollutants from anaerobic environments are available. Mechanical removal of contaminated sediment (dredging), removal of the overlying water body and subsequent physical removal of contaminated sediment (dry excavation), covering the contaminated sediment with fresh material (capping), and complete isolation of a contaminated area with containment barriers are associated with limited success (Perelo 2010). Chemical remediation options such as oxidation with H_2O_2, modified Fenton's reagent, activated sodium persulfate, potassium permanganate, and their combinations were shown to be effective in the removal of PAHs from contaminated sediments (Ferrarese et al. 2008). The practicability of physical and chemical treatment technologies is questioned due to cost, limited efficiency, technical complexity, destruction of habitats, and increased exposure to site workers and native organisms.

Bioremediation approaches offer several advantages over physical and chemical remediation options. But, investigations on the feasibility of laboratory- and field-scale bioremediation of PAHs from anaerobic environments are limited. Some studies suggested biostimulation (enhanced removal of contaminants following the addition of electron acceptors and/or nutrients) as a successful option for remediation of PAHs. For instance, nitrate addition in nitrate-deficient contaminated sediments resulted in significant removal of PAHs (Murphy et al. 1995; Rockne and Strand 2001; Tang et al. 2005; Yang et al. 2013). Similarly, sulfate addition in contaminated sediments promoted biodegradation of naphthalene, 2-MN, and phenanthrene (Rothermich et al. 2002; Tang et al. 2005). On the other hand, the arbitrary addition of electron acceptors appeared to be futile. Johnson and Ghosh (1998) compared the effect of nitrate and sulfate addition to dredged contaminated sediments. The sediments were rich in sulfate and supported appreciable anaerobic degradation of PAHs without any amendment. Addition of sulfate further enhanced PAHs degradation; however, nitrate addition did not have any stimulatory effect. Likewise, induction of methanogenesis by the addition of dextrin did not promote PAHs degradation. Similarly, Mn(IV) addition to contaminated sediment, where sulfate was the dominant electron acceptor, inhibited PAHs biodegradation (Li et al. 2011). Determination of dominant electron-accepting process in contaminated anaerobic environments, demonstration of the presence of requisite microorganisms, and laboratory-scale investigation into the efficacy of electron acceptor(s) seem to be critical steps in the process of anaerobic remediation through biostimulation.

Although laboratory-scale demonstrations of anaerobic PAHs degradation under various reducing conditions by enrichment and pure cultures are available, evidence suggesting their role in the degradation of PAHs from contaminated environments is still lacking. Future research should focus on evaluating the efficacy of PAHs-degrading cultures from real contaminated samples. The direct application of microorganisms as bioaugmentation agents also require site characterization and understanding of the degradation processes. Further research must also be directed toward feasibility assessment to establish the chance of success, effectiveness, and applicability. Moreover, laboratory investigations should focus on establishing the ability of native microorganisms to degrade PAHs from the anaerobic environments, deciphering the degradation mechanisms, identification of influencing parameters, and demonstration of bioremediation in laboratory-scale bioreactors. Behavior of anaerobic PAHs-degrading bacteria in the presence of mixed contaminants, for example, PAHs + heavy metals + cyanides, should be investigated. As strict anaerobes are sensitive to atmospheric oxygen, inoculation of the contaminated sites with microorganisms would also require special techniques. In this direction, facultative anaerobes would offer more flexibility in terms of oxygen sensitivity and inoculation techniques.

18 Conclusion

Field- and laboratory-scale investigations have provided ample evidence that LMW PAHs are biodegradable under various anaerobic conditions. Besides, anaerobic degradation of 4–5 ring PAHs, for example, BaP, is also known for some facultative anaerobes. However, most of the carcinogenic PAHs are HMW compounds and have more compact ring system compared to the LMW congeners. Still, there is a dearth of evidence of biodegradability of HMW PAHs, especially in strictly anaerobic sulfate-reducing and methanogenic conditions. Most of the existing literature shows anaerobic biodegradation in enrichment cultures, and very few pure cultures have been isolated. As the biomarker-based in situ monitoring depends upon the knowledge on biochemical transformation pathways, anaerobic PAHs-degrading pure culture isolation should receive due attention from concerned researchers. Factors that affect anaerobic PAHs degradation are still poorly understood. In many real contaminated environments, PAHs exist with other co-contaminants such as heavy metals, BTEX compounds, cyanides, and phenolics. Effect of co-contaminants is almost neglected in anaerobic PAHs degradation studies.

Furthermore, interactions among degrading microorganisms in anaerobic environments should be investigated, as the natural environment is an interactive and interdependent system regarding niche and nutrient cycling. Biochemistry of naphthalene and 2-MN degradation in SRB is now known. The mechanisms may not necessarily be the same for the other reducing conditions. It is especially relevant for facultative anaerobes that can switch among aerobic, hypoxic, and anaerobic modes. Thus, future studies should focus on elucidating the mechanism of PAHs degradation in both facultative anaerobic and strictly anaerobic bacteria. Identification of HMW PAHs degradation metabolites will also guide to develop tools for contamination monitoring. Information on the suitability of bioremediation, for instance, nutrient amendment or bioaugmentation, in the restoration of PAHs-impacted anaerobic environments should come from field-scale investigations. The field of anaerobic degradation of PAHs is about to pass its infancy, and it's now expanding reasonably well. Now it is our time to learn how to translate the ever-growing knowledge into remediation planning.

19 Summary

Many natural environments such as subsurface soil, groundwater, freshwater, and marine sediment and sludge are devoid of oxygen, i.e., anaerobic. Feasibility of degradation of hazardous polycyclic aromatic hydrocarbons (PAHs) in anaerobic environment was questioned for a long time. Thermodynamically, anaerobic biodegradation of PAHs under different reducing conditions is feasible despite lower energy yield compared to the aerobic process. So far, degradation of PAHs by facultative and strict anaerobic bacteria and archaea under nitrate-, sulfate-, and

iron-reducing and methanogenic conditions have been reported. But, experimental evidence of high-molecular-weight (HMW) PAHs degradation is still lacking. Metabolic pathways for low-molecular-weight (LMW) naphthalene and 2-methylnaphthalene (2-MN) in SRB have been well investigated. In SRB, naphthalene is activated by carboxylation at C2 position, whereas 2-MN is activated by a fumarate addition reaction. Subsequently, anaerobic bacteria employ ring reductases system to overcome the resonance energy of PAHs. Striking dissimilarities between degradation pathways of LMW PAHs and HMW PAHs suggest that the anaerobic degradation mechanisms are either organism-specific, reducing condition-specific or substrate-specific, or all. Because of the limited understanding of anaerobic PAHs metabolism, in situ diagnosis of the impacted environment based on metabolite profiling is still underdeveloped. PAHs exert a selection pressure on the anaerobic microbial community that is often reflected in marked change in abundance, diversity, and function. Inorganic electron acceptor amendment could be a viable method for enhancing anaerobic biodegradation of PAHs. Further investigations on anaerobic degradation of PAHs, especially HMW members, under different redox conditions are crucial to understand the natural attenuation process and to develop approaches for remediation of anaerobic environments contaminated with PAHs.

Acknowledgment Kartik Dhar is grateful to the University of Newcastle for UNIPRS and UNRS central scholarship and to the University of Chittagong, Chittagong 4331, Bangladesh, for granting study leave.

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Abdel-Shafy HI, Mansour MSM (2016) A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egypt J Pet 25:107–123

Abu Laban N, Selesi D, Rattei T, Tischler P, Meckenstock RU (2010) Identification of enzymes involved in anaerobic benzene degradation by a strictly anaerobic iron-reducing enrichment culture. Environ Microbiol 12:2783–2796

Achten C, Hofmann T (2009) Native polycyclic aromatic hydrocarbons (PAH) in coals – a hardly recognized source of environmental contamination. Sci Total Environ 407:2461–2473

Acosta-González A, Marqués S (2016) Bacterial diversity in oil-polluted marine coastal sediments. Curr Opin Biotechnol 38:24–32

Agarry SE, Owabor CN (2011) Anaerobic bioremediation of marine sediment artificially contaminated with anthracene and naphthalene. Environ Technol 32:1375–1381

Aitken CM, Jones DM, Larter S (2004) Anaerobic hydrocarbon biodegradation in deep subsurface oil reservoirs. Nature 431:291

Akunna JC, Bizeau C, Moletta R (1993) Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol. Water Res 27:1303–1312

Al-Bashir B, Cseh T, Leduc R, Samson R (1990) Effect of soil/contaminant interactions on the biodegradation of naphthalene in flooded soil under denitrifying conditions. Appl Microbiol Biotechnol 34:414–419

Alejandro A-G, Ramon R-M, Silvia M (2013) Characterization of the anaerobic microbial community in oil-polluted subtidal sediments: aromatic biodegradation potential after the Prestige oil spill. Environ Microbiol 15:77–92

Allamandola L, Tielens A, Barker J (1989) Interstellar polycyclic aromatic hydrocarbons - the infrared emission bands, the excitation/emission mechanism, and the astrophysical implications. Astrophys J Suppl Ser 71:733–775

Ambrosoli R, Petruzzelli L, Minati JL, Marsan FA (2005) Anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions. Chemosphere 60:1231–1236

Anderson RT, Lovley DR (1999) Naphthalene and benzene degradation under Fe(III)-reducing conditions in petroleum-contaminated aquifers. Biorem J 3:121–135

Anderson RT, Lovley DR (2000) Anaerobic bioremediation of benzene under sulfate-reducing conditions in a petroleum-contaminated aquifer. Environ Sci Technol 34:2261–2266

Andersson JT, Achten C (2015) Time to say goodbye to the 16 EPA PAHs? Toward an up-to-date use of PACs for environmental purposes. Polycyc Aromat Compd 35:330–354

Annweiler E, Materna A, Safinowski M, Kappler A, Richnow HH, Michaelis W, Meckenstock RU (2000) Anaerobic degradation of 2-methylnaphthalene by a sulfate-reducing enrichment culture. Appl Environ Microbiol 66:5329–5333

Annweiler E, Michaelis W, Meckenstock RU (2002) Identical ring cleavage products during anaerobic degradation of naphthalene, 2-methylnaphthalene, and tetralin indicate a new metabolic pathway. Appl Environ Microbiol 68:852–858

Arthur EL, Rice PJ, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR (2005) Phytoremediation – an overview. Crit Rev Plant Sci 24:109–122

Azuma H, Toyota M, Asakawa Y, Kawano S (1996) Naphthalene – a constituent of *Magnolia* flowers. Phytochemistry 42:999–1004

Bach Q-D, Kim S-J, Choi S-C, Oh Y-S (2005) Enhancing the intrinsic bioremediation of PAH-contaminated anoxic estuarine sediments with biostimulating agents. J Microbiol 43:319–324

Bandowe BA, Meusel H (2017) Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment - a review. Sci Total Environ 581-582:237–257

Barton LL, Fauque GD (2009) Biochemistry, physiology and biotechnology of sulfate-reducing bacteria. Adv Appl Microbiol 68:41–98

Bauer JE, Capone DG (1985) Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments. Appl Environ Microbiol 50:81–90

Bedessem ME, Swoboda-Colberg NG, Colberg PJ (1997) Naphthalene mineralization coupled to sulfate reduction in aquifer-derived enrichments. FEMS Microbiol Lett 152:213–218

Beller HR, Spormann AM (1997) Anaerobic activation of toluene and *o*-xylene by addition to fumarate in denitrifying strain T. J Bacteriol 179:670–676

Berdugo-Clavijo C, Dong X, Soh J, Sensen CW, Gieg LM (2012) Methanogenic biodegradation of two-ringed polycyclic aromatic hydrocarbons. FEMS Microbiol Ecol 81:124–133

Bergmann F, Selesi D, Weinmaier T, Tischler P, Rattei T, Meckenstock RU (2011a) Genomic insights into the metabolic potential of the polycyclic aromatic hydrocarbon degrading sulfate-reducing *Deltaproteobacterium* N47. Environ Microbiol 13:1125–1137

Bergmann FD, Selesi D, Meckenstock RU (2011b) Identification of new enzymes potentially involved in anaerobic naphthalene degradation by the sulfate-reducing enrichment culture N47. Arch Microbiol 193:241–250

Biegert T, Fuchs G, Heider J (1996) Evidence that anaerobic oxidation of toluene in the denitrifying bacterium *Thauera aromatica* is initiated by formation of benzylsuccinate from toluene and fumarate. Eur J Biochem 238:661–668

Blumer M (1976) Polycyclic aromatic compounds in nature. Sci Am 234:34–45

Blumer M, Youngblood WW (1975) Polycyclic aromatic hydrocarbons in soils and recent sediments. Science 188:53–55

Boll M (2005) Dearomatizing benzene ring reductases. J Mol Microbiol Biotechnol 10:132–142

Boll M, Fuchs G (1995) Benzoyl-coenzyme a reductase (dearomatizing), a key enzyme of anaerobic aromatic metabolism: ATP dependence of the reaction, purification and some properties of the enzyme from *Thauera aromatica* strain K172. Eur J Biochem 234:921–933

Boll ES, Christensen JH, Holm PE (2008) Quantification and source identification of polycyclic aromatic hydrocarbons in sediment, soil, and water spinach from Hanoi, Vietnam. J Environ Monit 10:261–269

Borneff J, Selenka F, Kunte H, Maximos A (1968) Experimental studies on the formation of polycyclic aromatic hydrocarbons in plants. Environ Res 2:22–29

Breese K, Boll M, Alt-Morbe J, Schagger H, Fuchs G (1998) Genes coding for the benzoyl-CoA pathway of anaerobic aromatic metabolism in the bacterium *Thauera aromatica*. Eur J Biochem 256:148–154

Brune A, Frenzel P, Cypionka H (2000) Life at the oxic–anoxic interface: microbial activities and adaptations. FEMS Microbiol Rev 24:691–710

Burdige DJ (2007) Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets? Chem Rev 107:467–485

Burland SM, Edwards EA (1999) Anaerobic benzene biodegradation linked to nitrate reduction. Appl Environ Microbiol 65:529–533

Cabrerizo A, Galbán-Malagón C, Del Vento S, Dachs J (2014) Sources and fate of polycyclic aromatic hydrocarbons in the Antarctic and Southern Ocean atmosphere. Glob Biogeochem Cycles 28:1424–1436

Caldwell ME, Suflita JM (2000) Detection of phenol and benzoate as intermediates of anaerobic benzene biodegradation under different terminal electron-accepting conditions. Environ Sci Technol 34:1216–1220

Callaghan AV (2013) Metabolomic investigations of anaerobic hydrocarbon-impacted environments. Curr Opin Biotechnol 24:506–515

Canfield DE, Thamdrup B, Hansen JW (1993) The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction. Geochim Cosmochim Acta 57:3867–3883

Capone DG, Kiene RP (1988) Comparison of microbial dynamics in marine and freshwater sediments: contrasts in anaerobic carbon catabolism. Limnol Oceanogr 33:725–749

Cappenberg TE (1974) Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a fresh-water lake. I. Field observations. Antonie Van Leeuwenhoek 40:285–295

Cea-Barcia G, Carrère H, Steyer JP, Patureau D (2013) Evidence for PAH removal coupled to the first steps of anaerobic digestion in sewage sludge. Int J Chem Eng 2013:1–6. https://doi.org/10.1155/2013/450542

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. In: Rosenberg E (ed) Microorganisms to combat pollution. Springer, Dordrecht, pp 227–244. https://doi.org/10.1007/978-94-011-1672-5_16

Cerniglia C, Sutherland J (2010) Degradation of polycyclic aromatic hydrocarbons by fungi. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2079–2110

Cerniglia CE, Gibson DT, Van Baalen C (1979) Algal oxidation of aromatic hydrocarbons: formation of 1-naphthol from naphthalene by *Agmenellum quadruplicatum*, strain PR-6. Biochem Biophys Res Commun 88:50–58

Cerniglia CE, Van Baalen C, Gibson DT (1980) Metabolism of naphthalene by the cyanobacterium *Oscillatoria* sp., strain JCM. Microbiology 116:485–494

Chang W, Jones TN, Holoman TRP (2001) Anaerobic polycyclic aromatic hydrocarbon(PAH)-degrading enrichment cultures under methanogenic conditions. In: Sixth international in situ and on site bioremediation symposium, pp 205–209

Chang B, Shiung L, Yuan S (2002) Anaerobic biodegradation of polycyclic aromatic hydrocarbon in soil. Chemosphere 48:717–724

Chang B, Chang S, Yuan S (2003) Anaerobic degradation of polycyclic aromatic hydrocarbons in sludge. Adv Environ Res 7:623–628

Chang W, Um Y, Hoffman B, Holoman TRP (2005) Molecular characterization of polycyclic aromatic hydrocarbon (PAH)-degrading methanogenic communities. Biotechnol Prog 21:682–688

Chang W, Um Y, Holoman TRP (2006) Polycyclic aromatic hydrocarbon (PAH) degradation coupled to methanogenesis. Biotechnol Lett 28:425–430

Chang B-V, Chang IT, Yuan SY (2008) Anaerobic degradation of phenanthrene and pyrene in mangrove sediment. Bull Environ Contam Toxicol 80:145–149

Christensen N, Batstone DJ, He Z, Angelidaki I, Schmidt J (2004) Removal of polycyclic aromatic hydrocarbons (PAHs) from sewage sludge by anaerobic degradation. Water Sci Technol 50:237–244

Coates JD, Anderson RT, Lovley DR (1996) Oxidation of polycyclic aromatic hydrocarbons under sulfate-reducing conditions. Appl Environ Microbiol 62:1099–1101

Coates JD, Woodward J, Allen J, Philp P, Lovley DR (1997) Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. Appl Environ Microbiol 63:3589–3593

Coates JD, Chakraborty R, Lack JG, O'connor SM, Cole KA, Bender KS, Achenbach LA (2001) Anaerobic benzene oxidation coupled to nitrate reduction in pure culture by two strains of *Dechloromonas*. Nature 411:1039

Coates JD, Chakraborty R, McInerney MJ (2002) Anaerobic benzene biodegradation – a new era. Res Microbiol 153:621–628

Cohen M, Barlow MJ (2005) Polycyclic aromatic hydrocarbon emission bands in selected planetary nebulae: a study of the behaviour with gas phase C/O ratio. Mon Not R Astron Soc 362:1199–1207

Cunningham JA, Rahme H, Hopkins GD, Lebron C, Reinhard M (2001) Enhanced *in situ* bioremediation of BTEX-contaminated groundwater by combined injection of nitrate and sulfate. Environ Sci Technol 35:1663–1670

Daisy BH, Strobel GA, Castillo U, Ezra D, Sears J, Weaver DK, Runyon JB (2002) Naphthalene, an insect repellent, is produced by *Muscodor vitigenus*, a novel endophytic fungus. Microbiology 148:3737–3741

Davidova IA, Gieg LM, Duncan KE, Suflita JM (2007) Anaerobic phenanthrene mineralization by a carboxylating sulfate-reducing bacterial enrichment. ISME J 1:436–442

Deziel E, Paquette G, Villemur R, Lepine F, Bisaillon J (1996) Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. Appl Environ Microbiol 62:1908–1912

Didonato RJ Jr et al (2010) Genome sequence of the deltaproteobacterial strain Naphs2 and analysis of differential gene expression during anaerobic growth on naphthalene. PLoS One 5(11): e14072

Dolfing J, Xu A, Gray ND, Larter SR, Head IM (2009) The thermodynamic landscape of methanogenic PAH degradation. Microb Biotechnol 2:566–574

Dou J, Liu X, Ding A (2009) Anaerobic degradation of naphthalene by the mixed bacteria under nitrate reducing conditions. J Hazard Mater 165:325–331

Drew MC (1990) Sensing soil oxygen. Plant Cell Environ 13:681–693

Duran R, Cravo-Laureau C (2016) Role of environmental factors and microorganisms in determining the fate of polycyclic aromatic hydrocarbons in the marine environment. FEMS Microbiol Rev 40:814–830

Eberlein C, Estelmann S, Seifert J, Von Bergen M, Müller M, Meckenstock RU, Boll M (2013a) Identification and characterization of 2-naphthoyl-coenzyme A reductase, the prototype of a novel class of dearomatizing reductases. Mol Microbiol 88:1032–1039

Eberlein C, Johannes J, Mouttaki H, Sadeghi M, Golding BT, Boll M, Meckenstock RU (2013b) ATP-dependent/−independent enzymatic ring reductions involved in the anaerobic catabolism of naphthalene. Environ Microbiol 15:1832–1841

Edwards E, Wills L, Reinhard M, Grbić-Galić D (1992) Anaerobic degradation of toluene and xylene by aquifer microorganisms under sulfate-reducing conditions. Appl Environ Microbiol 58:794–800

Egland PG, Pelletier DA, Dispensa M, Gibson J, Harwood CS (1997) A cluster of bacterial genes for anaerobic benzene ring biodegradation. Proc Natl Acad Sci U S A 94:6484–6489

Eriksson M, Sodersten E, Yu Z, Dalhammar G, Mohn WW (2003) Degradation of polycyclic aromatic hydrocarbons at low temperature under aerobic and nitrate-reducing conditions in enrichment cultures from northern soils. Appl Environ Microbiol 69:275–284

Estelmann S, Blank I, Feldmann A, Boll M (2015) Two distinct old yellow enzymes are involved in naphthyl ring reduction during anaerobic naphthalene degradation. Mol Microbiol 95:162–172

Ezra D, Hess WM, Strobel GA (2004) New endophytic isolates of *Muscodor albus*, a volatile-antibiotic-producing fungus. Microbiology 150:4023–4031

Fernández P, Grimalt JO (2003) On the global distribution of persistent organic pollutants. Chimia 57:514–521

Ferrarese E, Andreottola G, Oprea IA (2008) Remediation of PAH-contaminated sediments by chemical oxidation. J Hazard Mater 152:128–139

Foght J (2008) Anaerobic biodegradation of aromatic hydrocarbons: pathways and prospects. J Mol Microbiol Biotechnol 15:93–120

Folwell BD, McGenity TJ, Price A, Johnson RJ, Whitby C (2016) Exploring the capacity for anaerobic biodegradation of polycyclic aromatic hydrocarbons and naphthenic acids by microbes from oil-sands-process-affected waters. Int Biodeterior Biodegrad 108:214–221

Frey PA, Reed GH (2012) The ubiquity of iron. ACS Chem Biol 7:1477–1481

Fuchs G, Boll M, Heider J (2011) Microbial degradation of aromatic compounds - from one strategy to four. Nat Rev Microbiol 9:803–816

Galushko A, Minz D, Schink B, Widdel F (1999) Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. Environ Microbiol 1:415–420

Gieg LM, Toth CR (2017) Signature metabolite analysis to determine *in situ* anaerobic hydrocarbon biodegradation. In: Boll M (ed) Anaerobic utilization of hydrocarbons, oils, and lipids, Handbook of hydrocarbon and lipid microbiology. Springer, Cham, pp 1–30

Gieg LM, Duncan KE, Suflita JM (2008) Bioenergy production via microbial conversion of residual oil to natural gas. Appl Environ Microbiol 74:3022–3029

Griebler C, Safinowski M, Vieth A, Richnow HH, Meckenstock RU (2004) Combined application of stable carbon isotope analysis and specific metabolites determination for assessing *in situ* degradation of aromatic hydrocarbons in a tar oil-contaminated aquifer. Environ Sci Technol 38:617–631

Grimmer G, Jacob J, Naujack K-W (1983) Profile of the polycyclic aromatic compounds from crude oils. Fresenius Z Anal Chem 314:29–36

Habe H, Omori T (2003) Genetics of polycyclic aromatic hydrocarbon metabolism in diverse aerobic bacteria. Biosci Biotechnol Biochem 67:225–243

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169:1–15

Harwood CS, Burchhardt G, Herrmann H, Fuchs G (1998) Anaerobic metabolism of aromatic compounds via the benzoyl-CoA pathway. FEMS Microbiol Rev 22:439–458

Hayakawa K (2018) Oil spills and polycyclic aromatic hydrocarbons. In: Hayakawa K (ed) Polycyclic aromatic hydrocarbons: environmental behavior and toxicity in East Asia. Springer, Singapore, pp 213–223. https://doi.org/10.1007/978-981-10-6775-4_16

Hayes LA, Nevin KP, Lovley DR (1999) Role of prior exposure on anaerobic degradation of naphthalene and phenanthrene in marine harbor sediments. Org Geochem 30:937–945

Haynes WM (2014) CRC handbook of chemistry and physics. CRC Press, Boca Raton

Hazen TC (2010) Cometabolic bioremediation. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2505–2514. https://doi.org/10.1007/978-3-540-77587-4_185

Himmelberg AM, Brüls T, Farmani Z, Weyrauch P, Barthel G, Schrader W, Meckenstock RU (2018) Anaerobic degradation of phenanthrene by a sulfate-reducing enrichment culture. Environ Microbiol 20:3589–3600

Hong Y-W, Yuan D-X, Lin Q-M, Yang T-L (2008) Accumulation and biodegradation of phenanthrene and fluoranthene by the algae enriched from a mangrove aquatic ecosystem. Mar Pollut Bull 56:1400–1405

IARC (2010) Some non-heterocyclic polycyclic aromatic hydrocarbons and some related exposures. IARC Monogr Eval Carcinog Risks Hum 92:1

Jahn MK, Haderlein SB, Meckenstock RU (2005) Anaerobic degradation of benzene, toluene, ethylbenzene, and *o*-xylene in sediment-free iron-reducing enrichment cultures. Appl Environ Microbiol 71:3355–3358

Joback KG, Reid RC (1987) Estimation of pure-component properties from group-contributions. Chem Eng Commun 57:233–243

Jobelius C, Ruth B, Griebler C, Meckenstock RU, Hollender J, Reineke A, Frimmel FH, Zwiener C (2010) Metabolites indicate hot spots of biodegradation and biogeochemical gradients in a high-resolution monitoring well. Environ Sci Technol 45:474–481

Johann H, Georg F (1997) Anaerobic metabolism of aromatic compounds. Eur J Biochem 243:577–596

Johnsen AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. Environ Pollut 133:71–84

Johnson K, Ghosh S (1998) Feasibility of anaerobic biodegradation of PAHs in dredged river sediments. Water Sci Technol 38:41–48

Juhasz AL, Naidu R (2000) Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int Biodeterior Biodegrad 45:57–88

Kaiho K (1994) Benthic foraminiferal dissolved-oxygen index and dissolved-oxygen levels in the modern ocean. Geology 22:719–722

Kanaly RA, Harayama S (2000) Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J Bacteriol 182:2059–2067

Karavalakis G, Deves G, Fontaras G, Stournas S, Samaras Z, Bakeas E (2010) The impact of soy-based biodiesel on PAH, nitro-PAH and oxy-PAH emissions from a passenger car operated over regulated and nonregulated driving cycles. Fuel 89:3876–3883

Karickhoff SW, Brown DS, Scott TA (1979) Sorption of hydrophobic pollutants on natural sediments. Water Res 13:241–248

Kim K-H, Jahan SA, Kabir E, Brown RJ (2013) A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int 60:71–80

Kirso U, Irha N (1998) Role of algae in fate of carcinogenic polycyclic aromatic hydrocarbons in the aquatic environment. Ecotoxicol Environ Saf 41:83–89

Kleemann R, Meckenstock RU (2011) Anaerobic naphthalene degradation by Gram-positive, iron-reducing bacteria. FEMS Microbiol Ecol 78:488–496

Kobayashi H, Rittmann BE (1982) Microbial removal of hazardous organic compounds. Environ Sci Technol 16:170A–183A

Kraft B, Tegetmeyer H, Sharma R, Klotz M, Ferdelman T, Hettich R, Geelhoed J, Strous M (2014) The environmental controls that govern the end product of bacterial nitrate respiration. Science 345:676–679

Kukučka P, Lammel G, Dvorská A, Klánová J, Möller A, Fries E (2010) Contamination of Antarctic snow by polycyclic aromatic hydrocarbons dominated by combustion sources in the polar region. Environ Chem 7:504–513

Kummel S et al (2015) Anaerobic naphthalene degradation by sulfate-reducing *Desulfobacteraceae* from various anoxic aquifers. FEMS Microbiol Ecol 91(3):fiv006

Kung JW et al (2009) Identification and characterization of the tungsten-containing class of benzoyl-coenzyme A reductases. Proc Natl Acad Sci U S A 106:17687–17692

Kuppusamy S, Thavamani P, Venkateswarlu K, Lee YB, Naidu R, Megharaj M (2017) Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: technological constraints, emerging trends and future directions. Chemosphere 168:944–968

Kuypers MMM, Marchant HK, Kartal B (2018) The microbial nitrogen-cycling network. Nat Rev Microbiol 16:263

Laflamme RE, Hites RA (1978) The global distribution of polycyclic aromatic hydrocarbons in recent sediments. Geochim Cosmochim Acta 42:289–303

Landrum P, Giesy J, Oris J, Allred P (1986) Photoinduced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms. In: Vandermuelen J, Hrudey S (eds) Oil in freshwater: chemistry, biology, countermeasure technology. Pergamon Press, Elmsford, pp 304–318

Langenhoff AAM, Zehnder AJB, Schraa G (1996) Behaviour of toluene, benzene and naphthalene under anaerobic conditions in sediment columns. Biodegradation 7:267–274

Latimer J, Zheng J (2003) The sources, transport and fate of PAHs in the marine environment. In: Douben P (ed) PAHs: an ecotoxicological perspective. Wiley, West Sussex, pp 7–53

Leduc R, Samson R, Al-Bashir B, Al-Hawari J, Cseh T (1992) Biotic and abiotic disappearance of four pah compounds from flooded soil under various redox conditions. Water Sci Technol 26:51–60

Lei A, Wong Y, Tam N (2002) Removal of pyrene by different microalgal species. Water Sci Technol 46:195–201

Li C-H, Zhou H-W, Wong Y-S, Tam NF-Y (2009) Vertical distribution and anaerobic biodegradation of polycyclic aromatic hydrocarbons in mangrove sediments in Hong Kong, South China. Sci Total Environ 407:5772–5779

Li CH, Ye C, Wong YS, Tam NF (2011) Effect of Mn(IV) on the biodegradation of polycyclic aromatic hydrocarbons under low-oxygen condition in mangrove sediment slurry. J Hazard Mater 190:786–793

Li C, Wong Y, Wang H, Tam N (2015) Anaerobic biodegradation of PAHs in mangrove sediment with amendment of $NaHCO_3$. J Environ Sci 30:148–156

Liang L, Song X, Kong J, Shen C, Huang T, Hu Z (2014) Anaerobic biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by a facultative anaerobe *Pseudomonas* sp. JP1. Biodegradation 25:825–833

Lima ALC, Farrington JW, Reddy CM (2005) Combustion-derived polycyclic aromatic hydrocarbons in the environment – a review. Environ Forensic 6:109–131

Lovley DR (2001) Anaerobes to the rescue. Science 293:1444–1446

Lovley DR, Lonergan DJ (1990) Anaerobic oxidation of toluene, phenol, and *p*-cresol by the dissimilatory iron-reducing organism, GS-15. Appl Environ Microbiol 56:1858–1864

Lovley DR, Woodward JC, Chapelle FH (1994) Stimulated anoxic biodegradation of aromatic hydrocarbons using Fe (III) ligands. Nature 370:128

Lovley DR, Coates JD, Woodward JC, Phillips E (1995) Benzene oxidation coupled to sulfate reduction. Appl Environ Microbiol 61:953–958

Lu X, Zhang T, Fang HH-P, Leung KM, Zhang G (2011) Biodegradation of naphthalene by enriched marine denitrifying bacteria. Int Biodeterior Biodegrad 65:204–211

Lu X-Y, Li B, Zhang T, Fang HH (2012) Enhanced anoxic bioremediation of PAHs-contaminated sediment. Bioresour Technol 104:51–58

Lundstedt S et al (2007) Sources, fate, and toxic hazards of oxygenated polycyclic aromatic hydrocarbons (PAHs) at PAH-contaminated sites. Ambio 36:475–485

Luo L, Wang P, Lin L, Luan T, Ke L, Tam NFY (2014) Removal and transformation of high molecular weight polycyclic aromatic hydrocarbons in water by live and dead microalgae. Process Biochem 49:1723–1732

Luo L, Lai X, Chen B, Lin L, Fang L, Tam NF, Luan T (2015) Chlorophyll catalyse the photo-transformation of carcinogenic benzo[a]pyrene in water. Sci Rep 5:12776

Ma B, He Y, Chen H-H, Xu J-M, Rengel Z (2010) Dissipation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere: synthesis through meta-analysis. Environ Pollut 158:855–861
Ma C, Wang Y, Zhuang L, Huang D, Zhou S, Li F (2011) Anaerobic degradation of phenanthrene by a newly isolated humus-reducing bacterium, Pseudomonas aeruginosa strain PAH-1. J Soils Sed 11:923–929
MacRae JD, Hall KJ (1998) Biodegradation of polycyclic aromatic hydrocarbons (PAH) in marine sediment under denitrifying conditions. Water Sci Technol 38:177–185
Majora DW, Mayfielda CI, Barkerb JF (1988) Biotransformation of benzene by denitrification in aquifer sand. Groundwater 26:8–14
Maliszewska-Kordybach B (1999) Sources, concentrations, fate and effects of polycyclic aromatic hydrocarbons (PAHs) in the environment. Part A: PAHs in air. Pol J Environ Stud 8:131–136
Margulis L, Sagan D (1997) Microcosmos: four billion years of microbial evolution. University of California Press, New York
Marozava S, Mouttaki H, Muller H, Abu Laban N, Probst AJ, Meckenstock RU (2018) Anaerobic degradation of 1-methylnaphthalene by a member of the *Thermoanaerobacteraceae* contained in an iron-reducing enrichment culture. Biodegradation 29:23–39
Martens CS, Val Klump J (1984) Biogeochemical cycling in an organic-rich coastal marine basin 4. An organic carbon budget for sediments dominated by sulfate reduction and methanogenesis. Geochim Cosmochim Acta 48:1987–2004
Martirani-Von Abercron S-M, Pacheco D, Benito-Santano P, Marín P, Marqués S (2016) Polycyclic aromatic hydrocarbon-induced changes in bacterial community structure under anoxic nitrate reducing conditions. Front Microbiol 7:1775
McInerney MJ, Sieber JR, Gunsalus RP (2009) Syntrophy in anaerobic global carbon cycles. Curr Opin Biotechnol 20:623–632
McNally DL, Mihelcic JR, Lueking DR (1998) Biodegradation of three-and four-ring polycyclic aromatic hydrocarbons under aerobic and denitrifying conditions. Environ Sci Technol 32:2633–2639
McNeely RN, Neimanis VP, Dwyer L (1979) Water quality sourcebook: a guide to water quality parameters. Waters Directorate, Water Quality Branch, Ottawa
Means JC, Wood SG, Hassett JJ, Banwart WL (1980) Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environ Sci Technol 14:1524–1528
Meckenstock RU, Mouttaki H (2011) Anaerobic degradation of non-substituted aromatic hydrocarbons. Curr Opin Biotechnol 22:406–414
Meckenstock RU, Annweiler E, Michaelis W, Richnow HH, Schink B (2000) Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. Appl Environ Microbiol 66:2743–2747
Meckenstock RU, Safinowski M, Griebler C (2004) Anaerobic degradation of polycyclic aromatic hydrocarbons. FEMS Microbiol Ecol 49:27–36
Meckenstock RU et al (2016) Anaerobic degradation of benzene and polycyclic aromatic hydrocarbons. J Mol Microbiol Biotechnol 26:92–118
Menzie CA, Potocki BB, Santodonato J (1992) Exposure to carcinogenic PAHs in the environment. Environ Sci Technol 26:1278–1284
Mihelcic JR, Luthy RG (1988a) Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. Appl Environ Microbiol 54:1182–1187
Mihelcic JR, Luthy RG (1988b) Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. Appl Environ Microbiol 54:1188–1198
Miki S, Uno S, Ito K, Koyama J, Tanaka H (2014) Distributions of polycyclic aromatic hydrocarbons and alkylated polycyclic aromatic hydrocarbons in Osaka Bay, Japan. Mar Pollut Bull 85:558–565
Miller JS, Olejnik D (2001) Photolysis of polycyclic aromatic hydrocarbons in water. Water Res 35:233–243
Miller MM, Wasik SP, Huang GL, Shiu WY, Mackay D (1985) Relationships between octanol-water partition coefficient and aqueous solubility. Environ Sci Technol 19:522–529

Morgan P, Watkinson RJ (1992) Factors limiting the supply and efficiency of nutrient and oxygen supplements for the *in situ* biotreatment of contaminated soil and groundwater. Water Res 26:73–78

Mouttaki H, Johannes J, Meckenstock RU (2012) Identification of naphthalene carboxylase as a prototype for the anaerobic activation of non-substituted aromatic hydrocarbons. Environ Microbiol 14:2770–2774

Mulas G, Malloci G, Joblin C, Toublanc D (2006) Estimated IR and phosphorescence emission fluxes for specific polycyclic aromatic hydrocarbons in the red rectangle. A&A 446:537–549

Müller JA, Schink B (2000) Initial steps in the fermentation of 3-hydroxybenzoate by *Sporotomaculum hydroxybenzoicum*. Arch Microbiol 173:288–295

Mulligan CN (2005) Environmental applications for biosurfactants. Environ Pollut 133:183–198

Murphy T, Moller A, Brouwer H (1995) *In situ* treatment of Hamilton Harbour sediment. Aquat Ecosyst Health Manag 4:195–203

Musat F et al (2009) Anaerobic degradation of naphthalene and 2-methylnaphthalene by strains of marine sulfate-reducing bacteria. Environ Microbiol 11:209–219

Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6:441

Nales M, Butler BJ, Edwards EA (1998) Anaerobic benzene biodegradation: a microcosm survey. Biorem J 2:125–144

Neilson AN (2013) PAHs and related compounds: chemistry, vol 3. Springer, Berlin

Neilson A, Allard A, Remberger M (1998) The handbook of environmental chemistry. Part J. PAHs and related compounds. Springer, Berlin

Nestler FHM (1974) Characterization of wood-preserving coal-tar creosote by gas-liquid chromatography. Anal Chem 46:46–53

Nielsen T, Ramdahl T, Bjørseth A (1983) The fate of airborne polycyclic organic matter. Environ Health Perspect 47:103

Nisbet EG, Sleep NH (2001) The habitat and nature of early life. Nature 409:1083

Northrop D, Simpson O, Mott N (1956) Electronic properties of aromatic hydrocarbons I. Electrical conductivity. Proc R Soc Lond A 234:124–135

O'Neil MJ (2013) The Merck index: an encyclopedia of chemicals, drugs, and biologicals. RSC Publishing, London

Ohlenbusch G, Zwiener C, Meckenstock RU, Frimmel FH (2002) Identification and quantification of polar naphthalene derivatives in contaminated groundwater of a former gas plant site by liquid chromatography–electrospray ionization tandem mass spectrometry. J Chromatogr 967:201–207

Ohura T (2007) Environmental behavior, sources, and effects of chlorinated polycyclic aromatic hydrocarbons. Sci World J 7:372–380

Oka AR, Phelps CD, Zhu X, Saber DL, Young L (2011) Dual biomarkers of anaerobic hydrocarbon degradation in historically contaminated groundwater. Environ Sci Technol 45:3407–3414

Okere U, Semple K (2012) Biodegradation of PAHs in 'pristine'soils from different climatic regions. J Bioremed Biodegr S1:006

Oko BJ, Tao Y, Stuckey DC (2017) Dynamics of two methanogenic microbiomes incubated in polycyclic aromatic hydrocarbons, naphthenic acids, and oil field produced water. Biotechnol Biofuels 10:1–13

Ou S, Zheng J, Zheng J, Richardson BJ, Lam PK (2004) Petroleum hydrocarbons and polycyclic aromatic hydrocarbons in the surficial sediments of Xiamen Harbour and Yuan Dan Lake, China. Chemosphere 56:107–112

Page DS, Brown JS, Boehm PD, Bence AE, Neff JM (2006) A hierarchical approach measures the aerial extent and concentration levels of PAH-contaminated shoreline sediments at historic industrial sites in Prince William Sound, Alaska. Mar Pollut Bull 52:367–379

Parisi VA, Brubaker GR, Zenker MJ, Prince RC, Gieg LM, Da Silva MLB, Alvarez PJJ, Suflita JM (2009) Field metabolomics and laboratory assessments of anaerobic intrinsic bioremediation of hydrocarbons at a petroleum-contaminated site. Microb Biotechnol 2:202–212

Peeters E (2011) The PAH hypothesis after 25 years. Proc Int Astron Union 7:149–161
Peng R-H et al (2008) Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiol Rev 32:927–955
Perelo LW (2010) In situ and bioremediation of organic pollutants in aquatic sediments. J Hazard Mater 177:81–89
Phelps CD, Battistelli J, Young L (2002) Metabolic biomarkers for monitoring anaerobic naphthalene biodegradation in situ. Environ Microbiol 4:532–537
Phelps CD, Kazumi J, Young LY (1996) Anaerobic degradation of benzene in BTX mixtures dependent on sulfate reduction. FEMS Microbiol Lett 145:433–437
Planavsky NJ et al (2014) Evidence for oxygenic photosynthesis half a billion years before the Great Oxidation Event. Nat Geosci 7:283
Qin W, Zhu Y, Fan F, Wang Y, Liu X, Ding A, Dou J (2017) Biodegradation of benzo(a)pyrene by *Microbacterium* sp. strain under denitrification: degradation pathway and effects of limiting electron acceptors or carbon source. Biochem Eng J 121:131–138
Qin W, Fan F, Zhu Y, Huang X, Ding A, Liu X, Dou J (2018) Anaerobic biodegradation of benzo (a)pyrene by a novel *Cellulosimicrobium cellulans* CWS2 isolated from polycyclic aromatic hydrocarbon-contaminated soil. Braz J Microbiol 49:258–268
Rabus R, Kube M, Heider J, Beck A, Heitmann K, Widdel F, Reinhardt R (2005) The genome sequence of an anaerobic aromatic-degrading denitrifying bacterium, strain EbN1. Arch Microbiol 183:27–36
Rafin C, Potin O, Veignie E, Sahraoui L-HA, Sancholle M (2000) Degradation of Benzo[a]Pyrene as sole carbon source by a non white rot fungus, *Fusarium solani*. Polycyc Aromat Compd 21:311–329
Ribeiro H et al (2018) Potential of dissimilatory nitrate reduction pathways in polycyclic aromatic hydrocarbon degradation. Chemosphere 199:54–67
Rochman CM, Manzano C, Hentschel BT, Simonich SLM, Hoh E (2013) Polystyrene plastic: a source and sink for polycyclic aromatic hydrocarbons in the marine environment. Environ Sci Technol 47:13976–13984
Rockne KJ, Strand SE (1998) Biodegradation of bicyclic and polycyclic aromatic hydrocarbons in anaerobic enrichments. Environ Sci Technol 32:3962–3967
Rockne KJ, Strand SE (2001) Anaerobic biodegradation of naphthalene, phenanthrene, and biphenyl by a denitrifying enrichment culture. Water Res 35:291–299
Rockne KJ, Chee-Sanford JC, Sanford RA, Hedlund BP, Staley JT, Strand SE (2000) Anaerobic naphthalene degradation by microbial pure cultures under nitrate-reducing conditions. Appl Environ Microbiol 66:1595–1601
Ron EZ, Rosenberg E (2002) Biosurfactants and oil bioremediation. Curr Opin Biotechnol 13:249–252
Ross AB et al (2002) Measurement and prediction of the emission of pollutants from the combustion of coal and biomass in a fixed bed furnace. Fuel 81:571–582
Rothermich MM, Hayes LA, Lovley DR (2002) Anaerobic, sulfate-dependent degradation of polycyclic aromatic hydrocarbons in petroleum-contaminated harbor sediment. Environ Sci Technol 36:4811–4817
Safinowski M, Meckenstock RU (2004) Enzymatic reactions in anaerobic 2-methylnaphthalene degradation by the sulphate-reducing enrichment culture N47. FEMS Microbiol Lett 240:99–104
Safinowski M, Meckenstock RU (2006) Methylation is the initial reaction in anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. Environ Microbiol 8:347–352
Safinowski M, Griebler C, Meckenstock RU (2006) Anaerobic cometabolic transformation of polycyclic and heterocyclic aromatic hydrocarbons: evidence from laboratory and field studies. Environ Sci Technol 40:4165–4173
Samanta SK, Singh OV, Jain RK (2002) Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation. Trends Biotechnol 20:243–248

Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. Microbiol Mol Biol Rev 61:262–280

Schöcke L, Schink B (1999) Energetics and biochemistry of fermentative benzoate degradation by *Syntrophus gentianae*. Arch Microbiol 171:331–337

Schoeny R, Cody T, Warshawsky D, Radike M (1988) Metabolism of mutagenic polycyclic aromatic hydrocarbons by photosynthetic algal species. Mutat Res 197:289–302

Schühle K, Gescher J, Feil U, Paul M, Jahn M, Schägger H, Fuchs G (2003) Benzoate-coenzyme A ligase from *Thauera aromatica*: an enzyme acting in anaerobic and aerobic pathways. J Bacteriol 185:4920–4929

Selesi D et al (2010) Combined genomic and proteomic approaches identify gene clusters involved in anaerobic 2-methylnaphthalene degradation in the sulfate-reducing enrichment culture N47. J Bacteriol 192:295–306

Sharak Genthner BR, Townsend GT, Lantz SE, Mueller JG (1997) Persistence of polycyclic aromatic hydrocarbon components of creosote under anaerobic enrichment conditions. Arch Environ Contam Toxicol 32:99–105

Shen G, Tao S, Wei S, Zhang Y, Wang R, Wang B, Li W, Shen H, Huang Y, Chen Y, Chen H, Yang Y, Wang W, Wang X, Liu W, Simonich SLM (2012) Emissions of parent, nitro, and oxygenated polycyclic aromatic hydrocarbons from residential wood combustion in rural China. Environ Sci Technol 46:8123–8130

Sims RC, Overcash MR (1983) Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Residue Rev 88:1–68

Sivaram AK, Logeshwaran P, Subashchandrabose SR, Lockington R, Naidu R, Megharaj M (2018) Comparison of plants with C3 and C4 carbon fixation pathways for remediation of polycyclic aromatic hydrocarbon contaminated soils. Sci Rep 8:2100

Skupinska K, Misiewicz I, Kasprzycka-Guttman T (2004) Polycyclic aromatic hydrocarbons: physicochemical properties, environmental appearance and impact on living organisms. Acta Pol Pharm 61:233–240

Smith MB, March J (2007) March's advanced organic chemistry: reactions, mechanisms, and structure. Wiley, Hoboken

Sporstol S, Gjos N, Lichtenthaler RG, Gustavsen KO, Urdal K, Oreld F, Skei J (1983) Source identification of aromatic hydrocarbons in sediments using GC/MS. Environ Sci Technol 17:282–286

Stein SE, Fahr A (1985) High-temperature stabilities of hydrocarbons. J Phys Chem 89:3714–3725

Stogiannidis E, Laane R (2015) Source characterization of polycyclic aromatic hydrocarbons by using their molecular indices: an overview of possibilities. Rev Environ Contam Toxicol 234:49–133

Subashchandrabose SR, Krishnan K, Gratton E, Megharaj M, Naidu R (2014) Potential of fluorescence imaging techniques to monitor mutagenic PAH uptake by microalga. Environ Sci Technol 48:9152–9160

Subashchandrabose SR, Logeshwaran P, Venkateswarlu K, Naidu R, Megharaj M (2017) Pyrene degradation by *Chlorella* sp. MM3 in liquid medium and soil slurry: possible role of dihydrolipoamide acetyltransferase in pyrene biodegradation. Algal Res 23:223–232

Sullivan ER, Zhang X, Phelps C, Young L (2001) Anaerobic mineralization of stable-isotope-labeled 2-methylnaphthalene. Appl Environ Microbiol 67:4353–4357

Sutherland JB, Freeman JP, Selby AL, Fu PP, Miller DW, Cerniglia CE (1990) Stereoselective formation of a K-region dihydrodiol from phenanthrene by *Streptomyces flavovirens*. Arch Microbiol 154:260–266

Sutton MA, Oenema O, Erisman JW, Leip A, van Grinsven H, Winiwarter W (2011) Too much of a good thing. Nature 472:159

Tang YJ, Carpenter S, Deming J, Krieger-Brockett B (2005) Controlled release of nitrate and sulfate to enhance anaerobic bioremediation of phenanthrene in marine sediments. Environ Sci Technol 39:3368–3373

Thauer RK, Jungermann K, Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol Rev 41:100–180
Thomas J, Ward C (1989) In situ biorestoration of organic contaminants in the subsurface. Environ Sci Technol 23:760–766
Tiedje JM, Sexstone AJ, Myrold DD, Robinson JA (1983) Denitrification: ecological niches, competition and survival. Antonie Van Leeuwenhoek 48:569–583
Tielens AG (2005) The physics and chemistry of the interstellar medium. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9780511819056
Tongpim S, Pickard MA (1999) Cometabolic oxidation of phenanthrene to phenanthrene *trans*-9, 10-dihydrodiol by *Mycobacterium* strain S1 growing on anthracene in the presence of phenanthrene. Can J Microbiol 45:369–376
Toth CRA, Berdugo-Clavijo C, O'Farrell CM, Jones GM, Sheremet A, Dunfield PF, Gieg LM (2018) Stable isotope and metagenomic profiling of a methanogenic naphthalene-degrading enrichment culture. Microorganisms 6(3):E65
Townsend GT, Prince RC, Suflita JM (2003) Anaerobic oxidation of crude oil hydrocarbons by the resident microorganisms of a contaminated anoxic aquifer. Environ Sci Technol 37:5213–5218
Trably E, Patureau D, Delgenes J (2003) Enhancement of polycyclic aromatic hydrocarbons removal during anaerobic treatment of urban sludge. Water Sci Technol 48:53–60
Tsai J-C, Kumar M, Lin J-G (2009) Anaerobic biotransformation of fluorene and phenanthrene by sulfate-reducing bacteria and identification of biotransformation pathway. J Hazard Mater 164:847–855
Tsibart A, Gennadiev A, Koshovskii T, Watts A (2014) Polycyclic aromatic hydrocarbons in post-fire soils of drained peatlands in western Meshchera (Moscow region, Russia). Solid Earth 5:1305–1317
Tu Q et al (2014) GeoChip 4: a functional gene-array-based high-throughput environmental technology for microbial community analysis. Mol Ecol Resour 14:914–928
Tuyen LH, Tue NM, Takahashi S, Suzuki G, Viet PH, Subramanian A, Bulbule KA, Parthasarathy P, Ramanathan A, Tanabe S (2014) Methylated and unsubstituted polycyclic aromatic hydrocarbons in street dust from Vietnam and India: occurrence, distribution and in vitro toxicity evaluation. Environ Pollut 194:272–280
Ulrich AC, Beller HR, Edwards EA (2005) Metabolites detected during biodegradation of 13C6-benzene in nitrate-reducing and methanogenic enrichment cultures. Environ Sci Technol 39:6681–6691
US EPA (1980) Ambient water quality criteria for polynuclear aromatic hydrocarbons. Office of Water Regulation and Standards, Washington. EPA 440/5–80–069
US EPA (1982) An exposure and risk assessment for benzo(a)pyrene and other polycyclic aromatic hydrocarbons. Vol. 1. Summary. United States Environmental Protection Agency, Washington. EPA-440/4-85-020-V1. 1982
US EPA (2008) Polycyclic aromatic hydrocarbons (PAHs). Office of the Solid Waste, United States Environmental Protection Agency, Washington. https://archive.epa.gov/epawaste/hazard/wastemin/web/pdf/pahs.pdf. Accessed 12 Nov 2018
Valerio F, Bottino P, Ugolini D, Cimberle MR, Tozzi GA, Frigerio A (1984) Chemical and photochemical degradation of polycyclic aromatic hydrocarbons in the atmosphere. Sci Total Environ 40:169–188
Vázquez-Duhalt R, Ayala M, Márquez-Rocha FJ (2001) Biocatalytic chlorination of aromatic hydrocarbons by chloroperoxidase of *Caldariomyces fumago*. Phytochemistry 58:929–933
Vogel TM, Grbic-Galic D (1986) Incorporation of oxygen from water into toluene and benzene during anaerobic fermentative transformation. Appl Environ Microbiol 52:200–202
Wan R, Zhang S, Xie S (2012) Microbial community changes in aquifer sediment microcosm for anaerobic anthracene biodegradation under methanogenic condition. J Environ Sci 24:1498–1503

Wang X-C, Zhang Y-X, Chen RF (2001) Distribution and partitioning of polycyclic aromatic hydrocarbons (PAHs) in different size fractions in sediments from Boston Harbor, United States. Mar Pollut Bull 42:1139–1149

Wania F, Mackay D (1993) Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio 22:10–18

Wania F, Mackay D (1995) A global distribution model for persistent organic chemicals. Sci Total Environ 160:211–232

Wania F, Mackay D (1996) Peer reviewed: tracking the distribution of persistent organic pollutants. Environ Sci Technol 30:390A–396A

Warshawsky D, Cody T, Radike M, Reilman R, Schumann B, LaDow K, Schneider J (1995) Biotransformation of benzo[a]pyrene and other polycyclic aromatic hydrocarbons and heterocyclic analogs by several green algae and other algal species under gold and white light. Chem Biol Interact 97:131–148

Warshawsky D, LaDow K, Schneider J (2007) Enhanced degradation of benzo[a]pyrene by *Mycobacterium* sp. in conjunction with green alga. Chemosphere 69:500–506

Wawrik B et al (2012) Field and laboratory studies on the bioconversion of coal to methane in the San Juan Basin. FEMS Microbiol Ecol 81:26–42

Wei C, Bandowe BAM, Han Y, Cao J, Zhan C, Wilcke W (2015) Polycyclic aromatic hydrocarbons (PAHs) and their derivatives (alkyl-PAHs, oxygenated-PAHs, nitrated-PAHs and azaarenes) in urban road dusts from Xi'an, Central China. Chemosphere 134:512–520

Weiner JM, Lauck TS, Lovley DR (1998) Enhanced anaerobic benzene degradation with the addition of sulfate. Biorem J 2:159–173

Weiss MC, Sousa FL, Mrnjavac N, Neukirchen S, Roettger M, Nelson-Sathi S, Martin WF (2016) The physiology and habitat of the last universal common ancestor. Nat Microbiol 1:16116

Weyrauch P, Zaytsev AV, Stephan S, Kocks L, Schmitz OJ, Golding BT, Meckenstock RU (2017) Conversion of *cis*-2-carboxycyclohexylacetyl-CoA in the downstream pathway of anaerobic naphthalene degradation. Environ Microbiol 19:2819–2830

White P, Claxton L (2004) Mutagens in contaminated soil: a review. Mutat Res 567:227–345

Widdel F, Bak F (1992) Gram-negative mesophilic sulfate-reducing bacteria. In: The prokaryotes. Springer, New York, pp 3352–3378

Wiktorska K, Misiewicz-Krzeminska I, Kasprzycka-Guttman T (2004) Polycyclic aromatic hydrocarbons: physicochemical properties, environmental appearance and impact on living organisms. Acta Pol Pharm 61(3):233–240

Wilcke W (2000) Synopsis polycyclic aromatic hydrocarbons (PAHs) in soil – a review. J Plant Nutr Soil Sci 163:229–248

Wild SR, Jones KC (1995) Polynuclear aromatic hydrocarbons in the United Kingdom environment: a preliminary source inventory and budget. Environ Pollut 88:91–108

Wilson SC, Jones KC (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ Pollut 81:229–249

Wischgoll S, Taubert M, Peters F, Jehmlich N, von Bergen M, Boll M (2009) Decarboxylating and nondecarboxylating glutaryl-coenzyme a dehydrogenases in the aromatic metabolism of obligately anaerobic bacteria. J Bacteriol 191:4401–4409

Wu RS (2002) Hypoxia: from molecular responses to ecosystem responses. Mar Pollut Bull 45:35–45

Xu M et al (2014) Elevated nitrate enriches microbial functional genes for potential bioremediation of complexly contaminated sediments. ISME J 8:1932

Xu M, He Z, Zhang Q, Liu J, Guo J, Sun G, Zhou J (2015) Responses of aromatic-degrading microbial communities to elevated nitrate in sediments. Environ Sci Technol 49:12422–12431

Yan Z, Zhang Y, Wu H, Yang M, Zhang H, Hao Z, Jiang H (2017) Isolation and characterization of a bacterial strain *Hydrogenophaga* sp. PYR1 for anaerobic pyrene and benzo[a]pyrene biodegradation. RSC Adv 7:46690–46698

Yang X, Ye J, Lyu L, Wu Q, Zhang R (2013) Anaerobic biodegradation of pyrene by *Paracoccus denitrificans* under various nitrate/nitrite-reducing conditions. Water Air Soil Pollut 224:1578

Yu H (2002) Environmental carcinogenic polycyclic aromatic hydrocarbons: photochemistry and phototoxicity. J Environ Sci Health C 20:149–183

Yuan SY, Chang BV (2007) Anaerobic degradation of five polycyclic aromatic hydrocarbons from river sediment in Taiwan. J Environ Sci Health B 42:63–69

Zhang Y, Tao S (2009) Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. Atmos Environ 43:812–819

Zhang X, Young LY (1997) Carboxylation as an initial reaction in the anaerobic metabolism of naphthalene and phenanthrene by sulfidogenic consortia. Appl Environ Microbiol 63:4759–4764

Zhang X, Sullivan ER, Young LY (2000) Evidence for aromatic ring reduction in the biodegradation pathway of carboxylated naphthalene by a sulfate reducing consortium. Biodegradation 11:117–124

Zhang S, Wang Q, Xie S (2012a) Molecular characterization of phenanthrene-degrading methanogenic communities in leachate-contaminated aquifer sediment. Int J Environ Sci Technol 9:705–712

Zhang S, Wang Q, Xie S (2012b) Stable isotope probing identifies anthracene degraders under methanogenic conditions. Biodegradation 23:221–230

Zhang T, Tremblay P-L, Chaurasia AK, Smith JA, Bain TS, Lovley DR (2013) Anaerobic benzene oxidation via phenol in *Geobacter metallireducens*. Appl Environ Microbiol 79:7800–7806

Zhou Z, Yao Y, Wang M, Zuo X (2017) Co-effects of pyrene and nitrate on the activity and abundance of soil denitrifiers under anaerobic condition. Arch Microbiol 199:1091–1101

Climate Change and Bivalve Mass Mortality in Temperate Regions

Tan Kar Soon and Huaiping Zheng

Contents

Abbreviations

OA	Ocean acidification
HABs	Harmful algal blooms
ROD	Roseovarius oyster disease
IPCC	Intergovernmental panel on climate change
USA	United States of America
UK	United Kingdom
OH^-	Hydroxide
CO_3^{2-}	Carbonate

T. K. Soon · H. Zheng (✉)
Key Laboratory of Marine Biotechnology of Guangdong Province, Shantou University, Shantou, China
e-mail: johnnytan_87@hotmail.com; hpzheng@stu.edu.cn

P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology, Volume 251*, Reviews of Environmental Contamination and Toxicology Volume 251,
https://doi.org/10.1007/398_2019_31

1 Introduction

One of the fastest-growing global food sectors is the bivalve aquaculture industry. Bivalves particularly oysters, mussels and clams are important sources of animal protein (Tan and Ransangan 2016a, b). Bivalve aquaculture represents 14–16% of the average per capita animal protein for 1.5 billion people and supports over 200,000 livelihoods, mostly in developing countries (FAO 2018). Most of the bivalves produced around the world (89%) are from aquaculture (FAO 2016). To date, mollusc aquaculture have accounted for 21.42% (17.14 million tonnes) of the total aquaculture production, with Asia being the largest contributor (92.27%) (FAO 2018).

Unfortunately, there are reported increasing worldwide mass mortality trends in cultured mussels (McFarland et al. 2016; Tan and Ransangan 2016c), clams (Ortega et al. 2016), cockles (Defeo et al. 2013), oysters (Vohmann et al. 2009) and scallops (Leverone et al. 2007), which affect all life stages of bivalve from larvae to juvenile and adults (Yurimoto et al. 2014; Malham et al. 2012). In fact, many more natural bivalve populations suffer from mass mortalities (Wootton et al. 2003), although such events are not always published in scientific literature. In general, bivalve mass mortalities can result from various external and/or internal physiological factors. Several causative factors are responsible for mass mortalities of bivalves, but episodes of most bivalve mass mortalities are triggered by the synergistic effects of two or more factors (Callaway et al. 2013; Malham et al. 2012). Climate change issues particularly global warming and ocean acidification are the two main potential threats that have been predicted to increase the frequency and intensity of bivalve mortality outbreaks.

It cannot be denied that the climate is warming as the result of excessive human activities through the combustion of fossil fuels since 1950 (Feely et al. 2004). Seawater warming driven by anthropogenic emission is expected to negatively impact bivalve aquaculture (Filgueira et al. 2016). However, the direct links between climate change drivers (mainly ocean warming and ocean acidification) and bivalve mass mortality outbreaks are not well understood. Both warming and acidification require special attention regarding their potential impacts to aquaculture. Therefore, there is the need for rigorous scientific reviews on the links between climate change and mass mortalities in bivalves. Such information will aid in establishing an aquaculture and fishery management plan to be implemented in both commercial fisheries and nature conservation. To our knowledge, this paper represents the first review that summarizes climate change-related factors that caused episodic marine bivalves mass mortalities in temperate regions. The term “mass mortalities” used in this paper refers to a sudden loss (within 30 days) of more than 30% of the bivalve stock (Soletchnik et al. 2007).

2 Climate Change

Over the past two-and-a-half centuries, emissions released into the atmosphere from the burning of wood and fossil fuels for energy have led to a dramatic increase in atmospheric CO_2 levels from approximately 280 ppm at pre-industrial to about 400 ppm in year 2010 (Hartmann et al. 2013; Feely et al. 2004). The accumulation of CO_2 traps heat in the atmosphere, resulting in atmosphere and the ocean warming. Significant warming of sea surface temperatures has been documented in Australia (Lough and Hobday 2011), North Atlantic and Pacific, including near US coasts since 1900 (Deser et al. 2010), where global mean sea temperature has increased by about 0.7°C in the last century (IPCC 2007) (Fig. 1). In estimates of future CO_2 levels, based on business as usual emission scenarios, the oceanic surface temperature could increase by >3°C by the year 2100 (IPCC 2007).

Even though global oceans have absorbed about 550 billion tons of anthropogenic carbon dioxide (CO_2) (Hartmann et al. 2013), this absorption of atmospheric CO_2 has increased ocean acidity (ocean acidification (OA)). Since the beginning of the Industrial Revolution, global mean sea surface pH has declined by 0.1 units (from 8.2 to 8.1), equivalent to a 30% increase in ocean acidity (Feely et al. 2009). Seawater acidification leads to a shift in inorganic carbon equilibrium towards higher bicarbonate and lower carbonate ions (CO_3^{2-}) concentrations (Sabine et al. 2004). Anthropogenic climate change is expected to cause further decrease in the global mean sea surface pH up to 0.32 units, which can result in an increase in ocean acidity by 150% in the year 2100 (IPCC 2007) and could reach pH 7.4 by the year 2300 (Gattuso and Hansson 2011).

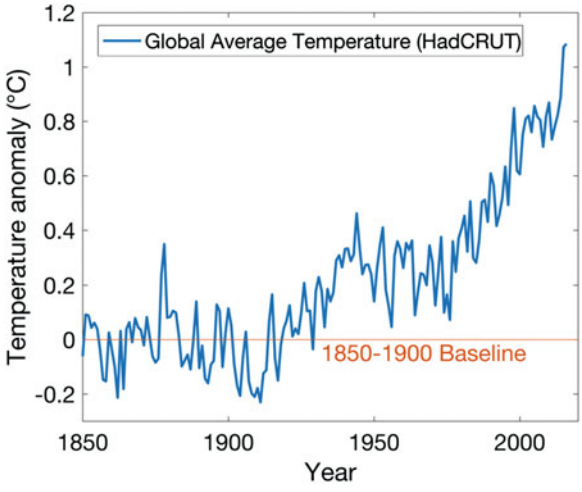

Fig. 1 Changes of global average temperature from pre-industrial to present (adopted from Google image)

3 Influence of Climate Change on Bivalve Mass Mortality

3.1 Warming Seawater Temperature and Dissolved Oxygen Depletion

In Arcachon Bay and Galicia, the presence of high organic matter accompanied by warm water caused mass mortality in juvenile cockles, *Cerastoderma edule* (Gonzalez and Perez Camacho 1984). High organic content in warm water promotes excessive microbial growth (Joint and Smale 2017), which consumes high levels of dissolved oxygen through microbial decomposition and results in dissolved oxygen depletion (Degerman et al. 2013). Similar observations have been reported in Uruguay and Argentina, where episodic mass mortalities of yellow clams, *Mesodesma mactroides*, along the sandy beaches of Uruguay and Argentina were associated with the warm seasons since the early 1990s (late spring to early summer) (Defeo et al. 2013; Fiori and Cazzaniga 1999). Twenty-two-year (from 1985 to 2007) observation provides a solid evidence that the warming of sea surface water due to anthropogenic climate change did not only lead to recurrence of mass mortality outbreaks but also decreased the abundance and individual size and caused shell abnormalities in the yellow clams along the sandy beaches of Uruguay (Ortega et al. 2016). In Puget Sound, Washington, and Tomales Bay, California, mass mortality of *Crassostrea gigas* occurred in summer 1998 during periods when water temperature elevated with corresponding low dissolved oxygen concentration (Cheney et al. 2000). In the year 2009, an unusual low river flow to Miño estuary, Spain, leads to dissolved oxygen depletion and high temperatures, resulting in mass mortality of *C. fluminea* (Ilarri et al. 2011).

The effects of elevated temperature on bivalve survival and energy metabolism are species specific, with some species particularly *C. virginica* (Ivanina et al. 2013) and *Mytilus galloprovincialis* (Gazeau et al. 2014) being highly sensitive to elevated temperatures. An experimental study showed that *C. virginica* experienced 65% mortality when exposed to temperature of 27°C for 15 weeks (Ivanina et al. 2013). Another experimental study has shown 100% mortality of adult mussels *My. galloprovincialis* when water temperature increased by 3°C in summer (Gazeau et al. 2014). Temperature increase negatively affects bioenergetics of bivalves leading to energy deficiency, affecting their growth, reproduction and immunity, which could lead to mortality (Ivanina et al. 2013). On the other hand, *C. fluminea* is relatively sensitive to hypoxia (Matthews and McMahon 1999), and massive die-off of *C. fluminea* is frequently reported in areas with low-level dissolved oxygen (Vohmann et al. 2009; Werner and Rothhauot 2008; Mouthon and Daufesne 2006).

3.2 *Open Windows of Opportunity for Toxins Producing Algae*

The effects of anthropogenic climate change include altered seasonal patterns, with longer duration of summer time conditions and corresponding shifts in the timing of spring and fall transitions (IPCC 2007). Biogeographic boundary shifts in phytoplankton populations due to warming seawater temperature have led to the poleward spread of harmful algal bloom (HAB) species (Tan and Ransangan 2017, 2016d; Thomas et al. 2012). For instance, *Dinophysis* blooms had never been reported in the United Kingdom before 1997; however, there have been multiple *Dinophysis* blooms across this region since then (Whyte et al. 2014). Another example of the global expansion of HABs is *Aureococcus anophagefferens* which had never been documented before 1985, but since then it has recurred in the United States and South Africa annually (Gobler et al. 2005). Based on historical environmental and phytoplankton data (1951–2000) and predicted future (2051–2100) ocean conditions, 74% of the phytoplankton taxa shifted poleward at the median speed of 12.9 km per decade (Barton et al. 2016). Another prediction study based on the global circulation model demonstrated that the oceanic warming of the North Atlantic since 1982 has significantly increased the potential mean growth rate and duration (lengthened by as much as 8 weeks) of *Alexandrium fundyense* and *D. acuminata* blooms in Gulf of Maine, waters surrounding the United Kingdom and coastal Norway (Gobler et al. 2017). There is therefore a growing concern about the effects of HABs upon shellfish resources, both in terms of seafood safety and the sustainability of shellfish industries (Tan and Ransangan 2015). HABs species mainly *Prorocentrum minimum* (Sellner et al. 1995), *Heterocapsa circularisquama* (Matsuyama et al. 1996) and *Aureococcus anophagefferens* (Bricelj and MacQuarrie 2007) have been associated with climate change and mass mortality episodes in bivalves.

The first record of bivalve mass mortality episodes associated with *Prorocentrum minimum* bloom was documented by Leibovitz et al. (1984). The incident of scallops *Argopecten irradians* massive die-off during *P. minimum* blooms was thought to be due to physical damage caused by the apical tooth, a sharp anterior spine on some *Prorocentrum* taxa (Leibovitz et al. 1984). In Chesapeake Bay, USA, mass mortalities of commercial hard clams (*Mercenaria mercenaria*) (Wikfors and Smolowitz 1993) and oyster (*C. virginica*) larvae (Luckenbach et al. 1993) were observed in the presence of *P. minimum* blooms, indicating high density of *P. minimum* may have adverse effects on the filtering and feeding systems of bivalves. Moreover, the *P. minimum* blooms also caused 100% mortality of juvenile oysters within 14 days and at 33% bloom density caused 43% mortality over 22 days (Luckenbach et al. 1993). The gill damage hypothesis had not been confirmed, and no follow-up studies of scallops in natural *P. minimum* blooms were conducted until the occurrence of mass mortality of wild eastern oyster, *C. virginica*, populations in Chesapeake Bay (Sellner et al. 1995). The *P. minimum* induces thrombosis throughout the vascular systems of bay scallops which indicate enterotoxin may be involved (Wikfors and Smolowitz 1993; Landsberg 2002). In bioassay experiments, 100% mortality of

juvenile scallops was found when exposed to *P. minimum* isolates from Chesapeake Bay tributaries which had confirmed that *P. minimum* caused poor larval development (Wikfors 2005) and can kill juvenile oysters by altering immune-system competence, thereby compromising disease resistance (Hegaret and Wikfors 2005).

The initial report pertaining to bivalve mass mortality associated with dinoflagellate, *Heterocapsa circularisquama*, was first reported in Uranouchi Bay, Japan, in 1988, which caused mass mortality of cultured Manila clam *Ruditapes philippinarum* (Matsuyama et al. 1995). In 1989, *H. circularisquama* caused blooms (maximum 2×10^4 cells/mL) in Fukuoka Bay and resulted in mass mortalities of pearl oysters *Pinctada fucata*, Pacific oysters *C. gigas*, Manila clams *R. philippinarum* and blue mussels *My. galloprovincialis* (Matsuyama 1999; Yamamoto and Tanaka 1990). In 1992, another massive bloom by *H. circularisquama* occurred between August and November in Ago Bay and caused mass mortality of cultured *Pinctada fucata* (Matsuyama et al. 1996). Since then, *H. circularisquama* blooms have spread and frequently caused mass mortalities to more than 19 natural and cultivated bivalve species in embayments of western Japan (Matsuyama 2012). The toxin produced by the dinoflagellate *H. circularisquama* is very specific, as it is only harmful to bivalves but harmless to other animals (Basti et al. 2009). *H. circularisquama* induce several deleterious effects on juveniles and adult bivalves, ranging from marked shrinkage of the mantle and gut discoloration (dark brown to beige) during the early phase of the bloom (Matsuyama et al. 1996), followed by behavioural change (Basti and Segawa 2010) and impairments of the basic physiological functions of feeding and respiration (Matsuyama 1999). Prior to death, bivalves exhibit an extreme retraction of their mantle edge and siphon, along with recurrent vomiting behaviour before initiating a closure reaction followed by paralysis and death (Basti and Segawa 2010). Mortality of bivalves generally occurred during early to middle periods of the bloom (Matsuyama et al. 1996).

Aureococcus anophagefferens is a pelagophyte that causes harmful brown tide blooms with densities exceeding 10^6 cells/mL for extended periods in estuaries in the eastern United States and South Africa (Gobler et al. 2005). Brown tides do not produce toxins that poison humans but have deleterious effects on bivalves. The first occurrence of bivalve mass mortality episode associated with *A. anophagefferens* took place in 1985. The brown tide (0.9 to 1.5×10^6 cells/mL) incident caused reproductive failure and mass mortalities (30 to 100%) of natural and transplanted mussels, *My. edulis*, in Narragansett Bay, United States (Tracey 1988). At the same time, the bay scallop, *Ar. irradians*, fishery in Great South Bay, New York, was collapsed due to two *A. anophagefferens* blooms in summers of 1985 and 1986, respectively (Bricelj and Kuenstner 1989). Peak density of the brown tide (10^6 cells/mL) coincided with *Ar. irradians* spawning season, thus leading to recruitment failure (Bricelj et al. 1987). In 2000, 67% mortality of juvenile *M. mercenaria* was noted during a 4-week brown tide with a cell abundance $>10^5$ cells/mL in Great South Bay (Greenfield and Lonsdale 2002). In 2002, Wazniak and Glibert (2004) found that growth rates of juvenile clams were significantly lower during a brown tide bloom in Maryland with a cell abundance of 10^8 cells/L compared to the period following its collapse. The factor of the

bivalve mortality is attributed to cessation of feeding and starvation. There is evidence that a toxin-like principle in the extracellular, polysaccharide-like layer of *A. anophagefferens* deters feeding in bivalves (Draper et al. 1990; Gainey and Shumway 1991). Feeding reduction by brown tide in adult bivalves was proposed to occur via the inhibition of gill lateral cilia which are responsible for generation of feeding currents (Gainey and Shumway 1991). A laboratory study showed that brown tide, *A. anophagefferens*, consistently inhibits the growth of veliger in a dose-dependent manner, where moderate densities of *A. anophagefferens* (3.5×10^5 cells/mL) were sufficient to dramatically reduce the feeding activity of juvenile *M. mercenaria* (Bricelj et al. 2001). Moreover, juvenile *M. mercenaria* (1 mm) that survived from severe *A. anophagefferens* bloom (8×10^5 cells/mL) for 2 weeks were completely unable to resume normal growth rate, but 20% of the larval population is able to resume normal growth rate if exposed to lower cell densities of $<4 \times 10^5$ cells/mL (for 2 weeks) (Bricelj and MacQuarrie 2007). In addition, exposure to $>2 \times 10^5$ cells/mL of *A. anophagefferens* is sufficient to cause permanent metamorphic failure to hard clam larval populations. These lead to extension of planktonic stage of the larvae, hence increasing mortality rate (Bricelj and MacQuarrie 2007).

3.3 Facilitating Deadly Pathogens Infestation

There is growing evidence that climate change may affect the burden of infectious diseases, potentially altering pathogens occurrence, distribution, severity or seasonal cycles, as well as altering the physiological state of bivalves and their susceptibility to pathogen infections (Harvell et al. 2009). Since the early 1990s, climate change has been a hot topic due to growing evidence that suggest that warming of seawater contributes to proliferation and spread of *Perkinsus marinus* (Cook et al. 1998) and OsHV-1 (Peeler et al. 2012) northward. Moreover, warming sea temperature is also believed to increase the prevalence of *Vibrio parahaemolyticus* infection in noble scallop, *Chlamys nobilis*, in southern coast of China and therefore consequences in scallops mass mortality outbreaks almost annually since 1998 (Lan et al. 2018). Laboratory studies revealed that warming seawater temperature affects some important functional responses of hemocytes in bivalves. Significant decrease in phagocytic activities has been observed in hemocytes of *C. gallina* (Monari et al. 2007) and *C. virginica* (Hégaret et al. 2003) when kept at 30°C and 28°C, respectively, for 7 days. In *C. farreri*, the percentage of phagocytic hemocytes was significantly lower at 28°C than those placed at 11°C and 23°C after 1-h stress application (Chen et al. 2007). The decrease in enzymatic and phagocytic activities of hemocytes at higher temperature is believed to be due to increase number of death cells (Gagnaire et al. 2006). Numerous pathogens are known to be responsible for bivalve mass mortality outbreaks. However, in the present review, only pathogens that caused deadly diseases associated to climate change are discussed as summarized in Table 1.

Table 1 Major deadly pathogens infestation associated with bivalve mass mortality in temperate regions

Pathogen	Host	Outbreak location	Temperature association	Year	References
Vibrio splendidus	*Crassostrea gigas*	Brittany, France	19–23°C	Mid-eighties	Deslou-Paoli et al. (1982)
		Ireland	21°C	2003	Malham et al. (2010)
Roseovarius crassostreae	*C. virginica*	Northern United States	>20°C	Summer 1988 and 1989	Bricelj et al. (1992)
		France		Summer of 1993	Renault et al. (2002)
Herpes-like viruses	*C. gigas*	France and New Zealand	>16°C	Summer of 1991	Nicolas et al. (1992) and Hine et al. (1992)
		French Atlantic coast		1993	Renault et al. (1994a)
Perkinsus marinus	*C. virginica*	Gulf of Mexico	>24°C	Mid 1940s	Mackin et al. (1950)
		Delaware Bay and Cape Cod		1990 through 1992	Ford (1996)
		Southern Maine		1995	Ford (1996)

3.3.1 *Vibrio splendidus*

Vibrio splendidus biovar II is the pathogen that is responsible for juvenile summer mortality (also known as bacillary necrosis disease), which was first reported to cause mass mortality in larvae of *M. mercenaria* (Guillard 1959; Tubiash et al. 1965). In summer 1979, the *V. splendidus* caused mass mortality in juvenile Pacific oysters, *C. gigas*, for the first time. Since then, this phenomenon was only found to be specific to juvenile Pacific oyster *C. gigas* and has been observed numerous times in different countries including Japan, France, United States, Europe and Brazil (Saulnier et al. 2010; Garnier et al. 2007). In Brittany (France), *V. splendidus* has repeatedly caused mass mortality of 60 to 100% and 10 to 80% to *C. gigas* spat and juvenile (6 to 12 months old, 5 to 40 mm in shell length), respectively, since the mid-eighties (Deslou-Paoli et al. 1982). Similar disease outbreaks had also occurred along the coast of Europe, where high but sporadic *C. gigas* spat mortality rates (60 to 100%) in both wild and cultured oysters have been observed during summer since 1991 (Goulletquer et al. 1998). A 4-year epidemiological survey (2003–2007) confirmed that the high prevalence of *V. splendidus* and *V. aestuarianus* was associated with mortality events in Pacific oysters in France (Saulnier et al. 2010).

In all these cases, mortalities occurred when the temperature rose above 18°C and during the reproduction period (Saulnier et al. 2010; Malham et al. 2010; Goulletquer et al. 1998; Deslou-Paoli et al. 1982).

Clinical signs frequently observed in mollusc larvae suffering from vibriosis outbreaks include bacterial swarms on the margins of the larvae, detachment of velum cells, colonization of the mantle, invasion of the internal cavity and subsequently a necrosis of soft tissues followed by larval mortality between day 12 to 19 after hatching (Lacoste et al. 2001; Nicolas et al. 1996). Summer mortality of *C. gigas* is the result of complex interaction between oysters (host), their surrounding environment and opportunistic pathogens (Huvet et al. 2004). Some factors have been shown either to initiate mortalities or to promote *V. splendidus* infestation. These stressful conditions including low dissolved oxygen, harmful algae, pollutants or toxic substances released by sediments could trigger mortality, whereas management practices, oyster genetic origin and phytoplankton quantity and quality might modulate the extent of mortality (Malham et al. 2010; Harvell et al. 2009; Li et al. 2007; Degremont et al. 2005; Cheney et al. 2000). In 2003, mass mortality of oysters occurred in Ireland, where combination of high temperature (21°C) and high nutrient level (15 μM phosphate, 278 μM nitrate, 5.14 μM nitrite) was contributed to the high oyster mortalities of >20% (Malham et al. 2010). Elevated temperature facilitates the proliferation and spread of *V. splendidus* once the critical temperature threshold of 19°C is exceeded (Harvell et al. 2009). At the same time, high temperature periods coincides with oyster spawning season, which further compromises oyster immunity as post-spawning oysters have a lower thermal tolerance and a reduced ability to withstand pathogen infections (Li et al. 2007). Relative contribution of each causative agent (water temperature, spawning status and pathogen infection) to oyster summer mortality is responsible for less than a 15% increase of mortality risk, but taken together, the risk of mortality can add up to more than 50% (Wendling and Wegner 2013).

3.3.2 *Roseovarius crassostreae*

Roseovarius crassostreae is a member of the *Roseobacter* clade of the marine α-proteobacteria that causes *Roseovarius* oyster disease (ROD) which is also called juvenile oyster disease (JOD) (Maloy et al. 2007). The first occurrence of bivalve mass mortality associated with *R. crassostreae* was in Oyster Bay and Fishers Island, Northern United States, where mass mortality (40 to 90%) of hatchery reared juvenile oyster, *C. virginica*, at mean shell heights ranging from 15 to 24 mm occurred in summers 1988 and 1989, respectively (Bricelj et al. 1992). Since then, *R. crassostreae* has affected nursery operations along the Northeast Atlantic coast of the United States, from Maine to New York (Bricelj et al. 1992; Davis and Barber 1994; Ford and Borrero 2001; Maloy et al. 2007). Similar mass mortality episodes have also been reported in France, where 80 to 90% of *C. virginica* spats reared in the French Research Institution for the Exploitation of the Sea (IFREMER) laboratories in La Tremblade (Charente Maritime, France) and Bouin (Vendee, France) experienced massive die-off during summer of 1993 (Renault et al. 2002).

R. crassostreae affects seed oysters with size less than 25 mm in shell height (Davis and Barber 1994). Epizootics typically occur in the late summer at moderate salinities and temperatures (Davis and Barber 1994; Bricelj et al. 1992). Initial signs of *R. crassostreae* infection include a drastic reduction in growth rates, fragile and uneven valve margins, cupping of the left valve, thin, watery tissues, and anomalous deposits of conchiolin (brown ring) on the inner shell surfaces (Ford and Borrero 2001; Bricelj et al. 1992). Mortalities usually occur within 1–2 weeks of disease onset, and losses may exceed 95%, especially among individuals <25 mm in shell height (Davis and Barber 1994; Bricelj et al. 1992).

Laboratory study reveals *R. crassostreae* produce tufts of polar fimbriae that may be involved in colonization of susceptible oysters (Boettcher et al. 2005). This hypothesis is supported by microscopic observation of *R. crassostreae* attached by their poles to the inner shell surfaces of affected individuals (Boardman et al. 2008). Once established, colonization by *R. crassostreae* progresses along the shell surfaces in a biofilm-like mode of growth (Boardman et al. 2008), which may help them evade host defences. The deposit may cover the entire mantle surface and the attachment of the adductor muscle to the shell, which resembles the brown ring symptom of Manila clam, *R. philippinarum* (Ford and Borrero 2001).

3.3.3 Herpes-Like Viruses

Herpes-like viral infection was first reported in eastern oyster, *C. virginica*, from the east coast of the United States in 1972 (Farley et al. 1972). Because of the morphological similarity, it was later classified to be a member of Herpesviridae. Afterwards, disease outbreaks associated with herpes-like virus were later reported in Pacific oysters, *C. gigas*. In the summer of 1991, mass mortality outbreaks (60–100%), which were associated with the detection of a herpes-like virus, were reported among larvae of hatchery-reared Pacific oyster, *C. gigas*, for the first time in France (Nicolas et al. 1992) and New Zealand (Hine et al. 1992). Subsequently, another mass mortality outbreak (80 to 90%) among 3- to 7-month-old Pacific oysters was reported in July 1993 along the French Atlantic coast, where the presence of herpes-like virus particles in the connective tissue of gills and mantle (Renault et al. 1994a) was evident. At the same time, sporadic high mortalities (90–100%) occurred among batches of *C. gigas* larvae in several French hatcheries. In the summer 2008, abnormal mortality rates ranging from 80 to 100% that affected only *C. gigas* were reported in France, with higher mortalities observed in 2009 and 2010 (Segarra et al. 2010). Moreover, the oyster herpes-like virus was also found associated with *C. gigas* mass mortalities in Ireland, Italy, the Netherlands, Spain, the United Kingdom, Australia, New Zealand and Korea, while in other areas such as Japan, the virus was detected but did not cause oyster mortalities (e.g. Japan) (Lynch et al. 2012). Other than in *C. gigas*, herpes-like viruses have been identified in many bivalve species, including the Pacific oyster *C. gigas*, the European oyster *Ostrea edulis*, the Antipodean flat oyster *O. angasi*, the Chilean oyster *Tiostrea chilensis*, the Manila clam *R. philippinarum*, the carpet shell clam *R. decussatus*, the

Portuguese oyster *C. angulata*, the Suminoe oyster *C. rivularis* and the French scallop *Pecten maximus* (Renault et al. 2012; Hine and Thorne 1997; Hine 1997; Renault et al. 1994a, b; Hine et al. 1992; Comps and Cochennec 1993).

Transmission electron microscopy analysis showed that *C. gigas* larvae exhibited generalized infections, whereas focal infections usually occurred in juveniles. Adult stages were less sensitive than younger stages. Infected larvae at 3- to 4-day age showed a significant reduction in feeding and swimming activities (Da Silva et al. 2008). Significant mortality occurred by day 6, with 100% mortality by days 8–10 in most batches (Renault et al. 1994b). The effects of the herpes-like viral infection on the hosts are manifested in velar and mantle lesions and the attitude of larvae to swim weakly in circles. Histologically, fibroblastic-like cells exhibited abnormal cytoplasmic basophilia and enlarged nuclei with marginated chromatin; other cell types including hemocytes and myocytes showed extensive chromatin condensation (Da Silva et al. 2008).

3.3.4 *Perkinsus marinus*

Perkinsus marinus is a parasite that caused Dermo disease, which is characterized by emaciation of the digestive gland in *C. virginica* and, to a lesser extent, in *C. gigas*, *C. rhizophorae* and *C. corteziensis* (Ford 2011). Adult oyster is most susceptible after spawning, and mortality increases with age and size (Lauckner 1983). The *Perkinsus marinus* (formerly *Dermocystidium marinum*) was first discovered in the Gulf of Mexico in mid-1940s when unusual mortalities of oysters were observed (Mackin et al. 1950). For many years, *Perkinsus marinus* was restricted primarily to the region extending from lower Chesapeake along the Southern United States and Gulf Coasts. In general, prevalence and infection intensity increase with temperature and salinity even when other factors have an influence in these parameters (Soniat 1996). Beginning in the mid-1980s, *Perkinsus marinus* outbreaks occurred progressively farther north, which coincides with the warming trend (Ford 1996). In 1990, an epizootic began in Delaware Bay. From 1990 to 1992, disease outbreaks occurred as far north as Cape Cod, and in 1995, oysters in Southern Maine were found infected. In addition, Delaware Bay *C. virginica* stocks suffered annual mortality ranging from <5% to 55% due to various mortality agents, with higher rates being induced by *Perkinsus marinus* (Bushek et al. 2012). The occurrence of *Perkinsus marinus* was then found along the southeast coast of the United States from Maine to Florida (Remacha-Trivino et al. 2008), along the Pacific coast of Mexico (Caceres-Martinez et al. 2016), in the Gulf of California (northwest Mexico) (Enriquez-Espinoza et al. 2010) and in Brazil (Queiroga et al. 2015).

Perkinsus marinus is transmitted directly from oyster to oyster (Hofmann et al. 2009), as viable *Perkinsus marinus* cells are released in host faeces or on the death of the host (Park et al. 2010) and are acquired through host feeding. Every life stage of host is susceptible to disease (Paynter et al. 2010). The pathogen infects and proliferates in the digestive epithelium, connective tissue of all organs and

hemocytes causing hemocytosis and tissue lysis with a consequent severe emaciation, with up to 80% mortality arrives based on environmental factors (Smolowitz 2013).

3.4 Ocean Acidification

Ocean acidification (OA) is a potential threat to marine ecosystems (Halpern et al. 2008). Although there are no evidence that the elevated acidity of seawater at the present level can trigger bivalve mass mortality outbreaks, the changes in seawater chemistry could cause various negative effects on marine organisms. Meta-analysis predicted overall strong negative impact of future OA on marine biodiversity, particularly bivalve calcifying organisms (Kroeker et al. 2010), by diminishing availability of carbonate minerals which could hamper the development of early life stages, the process of calcification, growth, byssus attachment and survival (Kroeker et al. 2013, Gazeau et al. 2014, O'Donnell et al. 2013). A laboratory study showed that at pH levels of 0.3 units lower (pH 7.8) than the current global seawater pH (pH 8.1), the Ca and Mg content of shells of *M. edulis* reduced by 24% and 77%, respectively (Li et al. 2015). At pH 7.7 (0.4 units lower than current global seawater pH), strawberry conch, *Strombus luhuanus*, population experienced 10% higher mortality with significant lower shell height and weight compared to control within 26-week exposure (Shirayama and Thornton 2005). At pH of 0.7 units lower (pH 7.4) than current global seawater pH, *M. edulis* and *C. gigas* showed reduced calcification rate by 25% and 10%, respectively (Gazeau et al. 2007), whereas Mediterranean mussel *My. galloprovincialis* and the striped venus clam *Chamelea gallina* demonstrated shell damage area of 35% and 11%, respectively, compared to no shell damage at current global seawater pH (pH 8.1) (Bressan et al. 2014). At the same pH value of 7.4, blood clam *Tegillarca granosa* was 2.5 times more susceptible to *Vibrio* pathogen infection (Zha et al. 2017).

OA is expected to change the speciation of a number of metal ions in seawater where metals that form strong complexes with OH^- and CO_3^{2-} will have a higher fraction in their free forms (Byrne 2002). Ocean acidification is expected to reduce the carbonate ion level but increases free Pb^{2+}, Cu^{2+}, Ni^{2+} and Fe^{2+} concentrations from 3%, 8%, 4% and 66% at present (pH 8.1) to 6%, 32%, 13% and 90% by the year 2250 (expected pH 7.4), respectively (Millero et al. 2009a). Moreover, OA will increase the adsorption of metals as organic material due to most organic particles in seawater is negatively charged (Millero et al. 2009b). Therefore, OA will increase the metal accumulation in the body of filter feeder bivalves through direct absorption and feeding on suspended organic materials, which will reduce the survival rate of bivalve larvae by reducing the metabolic processes and delaying their metamorphosis (Kroeker et al. 2013). Laboratory studies also suggested that lower pH of 7.8 and 7.4 cause 1.21 and 1.32 times increase, respectively, in Cd accumulation in

M. meretrix compared to control at pH 8.07 ± 0.05 (Shi et al. 2016). Some bivalve species such as *Modiolus philippinarum* are sensitive to metals and have relatively low LC50 values (0.023, 0.221, 2.876, 2.337 and 0.007 mg/L for Cu, Cd, Pb, Zn and Hg (Ramakritinan et al. 2012).

Although interactive effects of elevated temperature and acidity of seawater on marine molluscs are poorly understood, laboratory studies have predicted harmful consequences of these events. For example, a 15-week laboratory study showed that increase in temperature (from 22 to 27°C) or decrease in pH (from 8.14 to 7.95) alone did not significantly affect the shell harness of *C. virginica* and *M. mercenaria*, respectively. However, the combination of these factors leads to significant negative effects on biomineralization causing in 5–10% decrease in shell hardness in *C. virginica* and *M. mercenaria* (Ivanina et al. 2013). Another 8-week laboratory study of reared mussel *My. edulis* at different combinations of pH (from 8.1 to 7.8) and temperature (19.22 to 25°C) revealed that OA significantly decreased the net calcification rate, modified shell ultrastructure and altered amino acid content. Notably, warming seawater temperature enhanced the effects of OA on these parameters and significantly decreased the shell breaking force (Li et al. 2015). The negative effects of combine ocean acidification and warming events on juvenile and larvae bivalve are even detrimental. Laboratory study showed that OA and seawater warming significantly reduced growth, survival, filtration rates and strength of shells in juvenile eastern oyster *C. virginica*. Moreover, increases in temperature (24 to 28°C) and CO_2 concentration (250 to 750 ppm) supressed survival, growth and lipid synthesis of *M. mercenaria* and *Ar. irradians* larvae by 50 to 60%, 5 to 10% and 2 to 10%, respectively (Talmage and Gobler 2011).

4 Conclusions

The earth's climate is changing that enhances global warming, and anthropogenic ocean acidification is happening. Warming of sea surface temperature has been shown to contribute to bivalve mass mortality outbreaks mainly by altering the physiological state of bivalves and their susceptibility to stressors and open windows of opportunity for biotoxins producing algae and facilitating pathogen infestations. On the other hand, although the degree of ocean acidification at present does not cause bivalve mass mortality, it has reduced growth and calcification rates and caused shell dissolution particularly in bivalve larvae and juveniles. Scientific evidences also show that the interactive impact of seawater acidification and elevated temperature on bivalves is likely to have ecological and functional implications, where ocean warming and acidification may act synergistically to increase the intensity and frequency of bivalve mass mortality outbreaks in the near future. In this context, some questions remain to be addressed. A lot more synergistic effects of various stressors caused by climate change remain to investigate in bivalves at different life stages. Moreover, the connections between climate change and bivalve mass mortality in tropical and polar regions are yet to be reviewed. More

importantly, efforts aimed at developing effective mitigation strategies to minimize the impact of climate change on the bivalve production industry should become the global priority in the next millennium in order to address the pressing seafood security issues.

5 Summary

Seawater warming and acidification resulting from anthropogenic activities are increasing threats to marine ecosystems. The increasing worldwide bivalve mass mortality trends since 1960s could be associated with climate change. Although there are many documented bivalve mass mortality cases, this information is not well organized, and the connection between climate change drivers (mainly ocean warming and ocean acidification) and bivalve mass mortality outbreaks is not well understood. Both warming and acidification require special attention regarding their potential impacts to ecosystem and to aquaculture industry. Therefore, there is the need for rigorous scientific reviews on the connection between climate change and the mass mortality of bivalves. Such information will aid in establishing an aquaculture and fishery management plan to be implemented in both commercial fisheries and nature conservation. In general, scientific evidences show warming sea surface temperature has directly triggered bivalve mass mortality outbreaks by causing low dissolved oxygen, facilitate the proliferation and spread of deadly diseases and promote higher frequency and intensity of harmful algal blooms. Although the present OA has not directly caused bivalve mass mortality, scientific evidences highlighted that bivalves will experience a reduction in calcification, shell thickness and shell breaking force which could cause bivalves more susceptible to various stressors. Therefore efforts aimed at minimizing the impacts of climate change to the bivalve production industry should become the global priority in the next millennium to address the pressing seafood security issues.

Acknowledgements The present study was financially supported by the National Natural Science Foundation of China (31872563), National Key R&D Program of China (2018YFD0901400), China Modern Agro-industry Technology Research System (CARS-49) and Department of Education of Guangdong Province (2017KCXTD014), China. We are very grateful to Dr. Jude Juventus Aweya (Department of Biology, Shantou University) for proofreading and English editing.

References

Barton AD, Irwin AJ, Finkel ZV, Stock CA (2016) Anthropogenic climate change drives shift and shuffle in North Atlantic phytoplankton communities. Proc Natl Acad Sci U S A 113(11):2964–2969

Basti L, Segawa S (2010) Mortalities of the short-neck clam *Ruditapes philippinarum* induced by the toxic dinoflagellate *Heterocapsa circularisquama*. Fish Sci 76:625–631

Basti L, Nagai K, Shimasaki Y, Oshima Y, Honjo T, Segawa S (2009) Effects of the toxic dinoflagellate *Heterocapsa circularisquama* on the valve movement behaviour of the Manila clam *Ruditapes philippinarum*. Aquaculture 291:41–47

Boardman CL, Maloy AP, Boettcher KJ (2008) Localization of the bacterial agent of juvenile oyster disease (*Roseovarius crassostreae*) within affected eastern oysters (*Crassostrea virginica*). J Invertebr Pathol 97:150–158

Boettcher KJ, Geaghan KK, Maloy AP, Barber BJ (2005) Roseovarius crassostreae sp. nov., a member of the Roseobacter clade and the apparent cause of juvenile oyster disease (JOD) in cultured Eastern oysters. Int J Syst Evol Microbiol 55:1531–1537

Bressan M, Chinellato A, Munari M, Matozzo V, Manci A, Marceta T, Finos L, Moro I, Pastore P, Badocco D, Marin MG (2014) Does seawater acidification affect survival, growth and shell integrity in bivalve juveniles? Mar Environ Res 99:136–148

Bricelj VM, Kuenstner SH (1989) Effects of the "Brown tide" on the feeding physiology and growth of bay scallops and mussels. In: Cosper EM, Bricelj VM, Carpenter EJ (eds) Novel phytoplankton blooms. Coastal and estuarine studies (formerly Lecture Notes on Coastal and Estuarine Studies), vol 35. Springer, Berlin

Bricelj VM, MacQuarrie SP (2007) Effects of brown tide (*Aureococcus anophagefferens*) on hard clam *Mercenaria mercenaria* larvae and implications for benthic recruitment. Mar Ecol Prog Ser 331:147–159

Bricelj VM, Epp J, Malouf R, E. (1987) Intraspecific variation in reproductive and somatic growth cycles of bay scallops *Argopecten irradians*. Mar Ecol Prog Ser 36:123–137

Bricelj VM, Ford SE, Borrero FJ, Perkins FO, Rivara G, Hillman RE, Elston RA, Chang J (1992) Unexplained mortalities of hatchery-reared, juvenile oysters, *Crassostrea virginica* (Gmelin). J Shellfish Res 11:331–347

Bricelj VM, MacQuarrie SP, Schaffner RA (2001) Differential effects of *Aureococcus anophagefferens* isolates ("brown tide") in unialgal and mixed suspensions on bivalve feeding. Mar Biol 139:605–615

Bushek D, Ford SE, Burt I (2012) Long-term patterns of an estuarine pathogen along a salinity gradient. J Mar Res 70:225–251

Byrne RH (2002) Inorganic speciation of dissolved elements in seawater: the influence of pH on concentration ratios. Geochem Trans 3:11–16

Caceres-Martinez J, Madero-Lopez LH, Padilla-Lardizabal G, Vasquez-Yeomans R (2016) Epizootiology of *Perkinsus marinus*, parasite of the pleasure oyster *Crassostrea corteziensis*, in the Pacific coast of Mexico. J Invertebr Pathol 139:12–18

Callaway R, Burdon D, Deasey A, Mazik K, Elliott M (2013) The riddle of the sands: population dynamics provides clues to causes of high cockle mortality. J Appl Ecol 50:1050–1059

Chen M, Yang H, Delaporte M, Zhao S (2007) Immune condition of *Chlamys farreri* in response to acute temperature challenge. Aquaculture 271:479–487

Cheney DP, MacDonald BF, Elston RA (2000) Summer mortality of Pacific oysters *Crassostrea gigas* (Thunberg): initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. J Shellfish Res 19(1):353–359

Comps M, Cochennec N (1993) A herpes-like virus from the European oyster *Ostrea edulis* L. J Invertebr Pathol 62:201–203

Cook T, Folli M, Klinck J, Ford S, Miller J (1998) The relationship between increasing sea-surface temperature and the northward spread of *Perkinsus marinus* (Dermo) disease epizootics in oysters. Estuar Coast Shelf Sci 46:587–597

Da Silva PM, Renault T, Fuentes J, Villalba A (2008) Herpesvirus infection in European flat oysters *Ostrea edulis* obtained from brood stocks of various geographic origins and grown in Galicia (NW Spain). Dis Aquat Organ 78:181–188

Davis CV, Barber BJ (1994) Size-dependent mortality in hatchery-reared populations of oysters, *Crassostrea virginica*, Gmelin 1791, affected by juvenile oyster disease. J Shellfish Res 13:137–142

Defeo O, Castrejón M, Ortega L, Kuhn AM, Gutiérrez NL, Castilla JC (2013) Impacts of climate variability on Latin American small-scale fisheries. Ecol Soc 18:30

Degerman R, Dinasquet J, Riemann L, de Luna SS, Anderson A (2013) Effect of resource availability on bacterial community responses to increased temperature. Aquat Microb Ecol 68:131–142

Degremont L, Bedier E, Soletchnik P, Ropert M, Huvet A, Moal J, Samain JF, Boudry P (2005) Relative importance of family, site, and field placement timing on survival, growth, and yield of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). Aquaculture 249:213–229

Deser C, Phillips AS, Alexander MA (2010) Twentieth century tropical sea surface temperature trends revisited. Geophys Res Lett 37

Deslou-Paoli JM, Herl M, Berthome JP, Razel D, Garnier J (1982) Reproduction naturelle de *Crassostrea gigas* Thunberg dans le basin de Marennes-Oleron en 1979 et 1981: aspects biochimiques et energetiques. Rev Trav Inst Pech Marit 45:319–327

Draper C, Gainey L, Shumway S, Shapiro L (1990) Effects of Aureococcus anophagefferens ("brown tide") on the lateral cilia of 5 species of bivalve molluscs. In: Graneli E et al (eds) Toxic marine phytoplankton. Proceedings of the 4th international conference. Elsevier, New York, pp 128–131

Enriquez-Espinoza TL, Grijalva-Chon JM, Castro-Longoria R, Ramos-Paredes J (2010) *Perkinsus marinus* in *Crassostrea gigas* in the Gulf of California. Dis Aquat Organ 89:269–273

FAO (2016) The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome

FAO (2018) The State of World Fisheries and Aquaculture 2018: meeting the sustainable developing goals. Rome. Licence: CC BY-NC-SA 3.0 IGO

Farley CA, Banfield WG, Kasnic JRG, Foster WS (1972) Oyster herpes-type virus. Science 178:759–760

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305:362–366

Feely RA, Doney SC, Cooley SR (2009) Ocean acidification: present conditions and future changes in a high-CO_2 world. Oceanography 22:36–47

Filgueira R, Guyondet T, Comeau LA, Tremblay R (2016) Bivalve aquaculture-environment interactions in the context of climate change. Glob Chang Biol 22(12):3901–3913

Fiori S, Cazzaniga N (1999) Mass mortality of the yellow clam, *Mesodesma mactroides* (Bivalvia, Mesodesmatidae) in Monte Hermoso Beach, Argentina. Biol Conserv 89:305–309

Ford SE (1996) Range extension by the oyster parasite *Perkinsus marinus* into the northern US: response to climate change? J Shellfish Res 15:45–56

Ford SE (2011) Dermo disease of oysters caused by Perkinsus marinus. In: Ford SE (ed) ICES identification leaflets for diseases and parasites of fish and shellfish. ICES, Copenhagen

Ford SE, Borrero FJ (2001) Epizootiology and pathology of juvenile oyster disease in the Eastern oyster, *Crassostrea virginica*. J Invertebr Pathol 78(3):141–154

Gagnaire B, Frouin H, Moreau K, Thomas-Guyon H, Renault T (2006) Effects of temperature and salinity on haemocyte activities of the Pacific oyster, *Crassostrea gigas* (Thunberg). Fish Shellfish Immunol 20:536–547

Gainey LF, Shumway SE (1991) The physiological effect of *Aureococcus anophagefferens* ('brown tide') on the lateral cilia of bivalve mollusks. Biol Bull 181:298–306

Garnier M, Labreuche Y, Garcia C, Robert M, Nicolas JL (2007) Evidence for the involvement of pathogenic bacteria in summer mortalities of the Pacific oyster *Crassostrea gigas*. Microb Ecol 53:187–196

Gattuso JP, Hansson L (eds) (2011) Ocean acidification. Oxford University Press, Oxford

Gazeau F, Quiblier C, Jansen JM, Gattuso JP, Middelburg JJ Heip CHR (2007) Impact of elevated CO_2 on shellfish calcification. Geophys Res Lett 34

Gazeau F, Alliouane S, Bock C, Bramanti L, Gentille M, Hirse T, Lopez M, Correa H-O, Pörtner HO, Ziveri P (2014) Impact of ocean acidification and warming on the Mediterranean mussel (*Mytilus galloprovincialis*). Front Mar Sci 1:62

Gobler CJ, Lonsdale DJ, Boyer GL (2005) A synthesis and review of causes and impact of harmful brown tide blooms caused by the alga, *Aureococcus anophagefferens*. Estuaries 28:726–749

Gobler CJ, Doherty OM, Hattenrath-Lehmann TK, Griffith AW, Kang Y, Litaker RW (2017) Ocean warming since 1982 has expanded the niche of toxic algal blooms in the North Atlantic and North Pacific oceans. Proc Natl Acad Sci U S A 114(19):4975–4980

Gonzalez R, Perez Camacho A (1984) El berberecho, *Cerastoderma edule* (L.) de Carril (Ria de Arosa) II: Reclutamiento, crecimiento, mortalidad natural y produccion. Actas IV Simposio Iberico do Estudos Do Benthos Marinho 2:223–244

Goulletquer P, Soletchnick P, Le Moine O, Razet D, Geairon P, Faury N, Taillade S (1998) Summer mortality of the Pacific cupped oyster *Crassostrea gigas* in the Bay of Marennes-Oléron (France). Ices Statutory Meeting, Population Biology, Mariculture Committee CM, CC 14-20

Greenfield DI, Lonsdale DJ (2002) Mortality and growth of juvenile hard clams *Mercenaria mercenaria* during brown tide. Mar Biol 141:1045–1050

Guillard RRL (1959) Further evidence of the destruction of bivalve larvae by bacteria. Biol Bull 117:258–266

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C et al (2008) A global map of human impact on marine ecosystems. Science 319:948–952

Hartmann DL, Klein Tank AMG, Rusticucci M (2013) Observation: atmosphere and surface. IPCC WGI AR5 (Report). p 198

Harvell D, Altizer S, Cattadori IM, Harrington L, Weil E (2009) Climate change and wildlife diseases: when does the host matter the most? Ecology 90:912–920

Hegaret H, Wikfors GH (2005) Effects of natural and field simulated blooms of the dinoflagellate *Prorocentrum minimum* upon hemocytes of eastern oysters, *Crassostrea virginica*, from two different populations. Harmful Algae 4:201–209

Hégaret H, Wikfors GH, Soudant P (2003) Flow-cytometric analysis of hemocytes from eastern oysters, *Crassostrea virginica*, subjected to a sudden temperature elevation: II. Hemocyte functions: aggregation, viability, phagocytosis and respiratory burst. J Exp Mar Biol Ecol 293:249–265

Hine PM (1997) Trends in research on diseases of bivalve mollusks. Bulletin of the European Association of Fish Pathologists 17:180–183

Hine PM, Thorne ET (1997) Replication of herpes-like viruses in haemocytes of adult flat oysters Ostrea angasi: an ultrastructural study. Dis Aquat Organ 29:189–196

Hine PM, Wesney B, Hay BE (1992) Herpesviruses associated with mortalities among hatchery-reared Pacific oysters, *Crassostrea gigas*. Dis Aquat Organ 12:135–142

Hofmann EE, Bushek D, Ford SE, Guo X (2009) Understanding how disease and environment combine to structure resistance in estuarine bivalve population. CCPO Publications. Paper 33

Huvet A, Herpin A, Degremont L, Labreuche Y, Samain JF, Cunningham C (2004) The identification of genes from the oyster *Crassostrea gigas* that are differentially expressed in progeny exhibiting opposed susceptibility to summer mortality. Gene 343(1):211–220

Ilarri M, Antunes C, Guilhermino L, Sousa R (2011) Massive mortality of the Asian clam *Corbicula fluminea* in a highly invaded area. Biol Invasions 13:277–280

IPCC (2007) Summary for policymakers. In: Solomon et al (eds) Climate change 2007: the physical science basis. Contribution of Working Group I to the fourth assessment report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge

Ivanina AV, Dickinson GH, Matoo OB, Bagwe R, Dickinson A, Beniash E, Sokolova IM (2013) Interactive effects of elevated temperature and CO_2 levels on energy metabolism and biomineralization of marine bivalves *Crassostrea virginica* and *Mercenaria mercenaria*. Comp Biochem Physiol A Mol Integr Physiol 166(1):101–111

Joint I, Smale DA (2017) Marie heat waves and optimal temperatures for microbial assemblage activity. FEMS Microbiol Ecol 93(2)

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol Lett 13:1419–1434

Kroeker KJ, Kordas RL, Crim RN, Hendriks IE, Ramajo L, Singh GS, Duarte CM, Gattuso JP (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob Chang Biol 19:1884–1896

Lacoste A, Jalabert F, Malham S, Cueff A, Gélébart F, Cordevant C, Lange M, Poulet SA (2001) A *vibrio splendidus* strain is associated with summer mortality of juvenile oysters *Crassostrea gigas* in the Bay of Morlaix (North Brittany, France). Dis Aquat Organ 46:139–145

Lan Y, Ye T, Xue Y, Liu H, Zhang H, Cheng D, Zhao M, Zhang Y, Li S, Ma H, Zheng H (2018) Physiological and immunological responses to mass mortality in noble scallop *Chlamys nobilis* cultured in Nan'ao waters of Shantou, China. Fish Shellfish Immunol 82:453–459

Landsberg JH (2002) The effects of harmful algal blooms on aquatic organisms. Rev Fish Sci 10:113–390

Lauckner G (1983) Diseases of mollusca: Bivalvia. In: Kinne O (ed) Diseases of marine animals volume II: introduction, Bivalvia to Scaphopoda. Biologische Anstalt Helgoland, Hamburg, pp 477–961

Leibovitz L, Schott EF, Karney RC (1984) Diseases of wild, captive and cultured scallops. J World Mariculture Soc 15(1):269–283

Leverone JR, Shumway SE, Blake NJ (2007) Comparative effects of the toxic dinoflagellate Karenia brevis on clearance rates in juvenile of four bivalve molluscs from Florida, USA. Toxicon 49(5):634–645

Li Y, Qin JG, Abbott CA, Li XX, Benkendorff K (2007) Synergistic impacts of heat shock and spawning on the physiology and immune health of *Crassostrea gigas*: an explanation for summer mortality in Pacific oysters. Am J Physiol 293:2353–2362

Li S, Liu C, Huang J, Liu Y, Zheng G, Xie L, Zhang R (2015) Interactive effects of seawater acidification and elevated temperature on biomineralization and amino acid metabolism in the mussel *Mytilus edulis*. J Exp Biol 218:3623–3631

Lough JM, Hobday AJ (2011) Observed climate change in Australian marine and freshwater environments. Mar Freshw Res 62:984–999

Luckenbach MW, Sellner KG, Shumway SE, Greene K (1993) Effects of two bloom-forming dinoflagellates, *Prorocentrum minimum* and *Gyrodinium uncatenum*, on the growth and survival of the eastern oyster, *Crassostrea virginica* (Gmelin 1791). J Shellfish Res 12:411–415

Lynch SA, Carlson J, Reilly AO, Cotter E, Culloty SC (2012) A previously undescribed ostreid herpesvirus 1 (OsHV-1) genotype detected in the Pacific oyster, *Crassostrea gigas*, in Ireland. Parasitology 139:1526–1532

Mackin JG, Owen HM, Collier A (1950) Preliminary note on the occurrence of a new protistan parasite, *Dermocystidium marinum* n. sp. in *Crassostrea virginica* (Gmelin). Science 111:328–329

Malham SK, Cotter E, O'Keeffe S, Lynch S, Culloty SC, King JW, Latchford JW, Beaumont AR (2010) Summer mortality of the Pacific oyster, *Crassostrea gigas*, in the Irish Sea: The influence of temperature and nutrients on health and survival. Aquaculture 287(1–2):128–138

Malham SK, Hutchinson TH, Longshaw M (2012) A review of the biology of European cockles (*Cerastoderma* spp.). J Marine Biol Assoc UK 92:1563–1577

Maloy AP, Ford SE, Karney RC, Boettcher KJ (2007) *Roseovarius crassostreae*, the etiological agent of Juvenile Oyster Disease (now to be known as *Roseovarius* Oyster Disease) in *Crassostrea virginica*. Aquaculture 269:71–83

Matsuyama Y (1999) Harmful effect of dinoflagellate *Heterocapsa cicularisquama* on shellfish aquaculture in Japan. Jpn Agr Res Q 33:283–293

Matsuyama Y (2012) Impacts of the harmful dinoflagellate *Heterocapsa circularisquama* bloom on shellfish aquaculture in Japan and some experimental studies on invertebrates. Harmful Algae 14:144–155

Matsuyama Y, Nagai K, Mizuguchi T, Fujiwara M, Ishimura M, Yamaguchi M, Uchida T, Honjo T (1995) Ecological features and mass mortality of pearl oysters during the red tide of Heterocapsa sp. in Ago Bay in 1992. Nippon Suisan Gakk 61:35–41

Matsuyama Y, Uchida T, Nagai K, Ishimura M, Nishimura A, Yamaguchi M, Honjo T (1996) Harmful and toxic algal blooms. In: Yasumoto T, Oshima Y, Fukuyo Y (eds) Intergovernmental Oceanographic Commission of UNESCO, pp 247–250

Matthews MA, McMahon RF (1999) Effects of temperature and temperature acclimation on survival of zebra mussel (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) under extreme hypoxia. J Moll Stud 65:317–325

McFarland K, Jean F, Thebault J, Volety AK (2016) Potential impacts of blooms of the toxic dinoflagellate *Karenia brevis* on the growth, survival and juvenile recruitment of the non-native green mussel *Perna viridis* in Southeastern United States. Toxicon 109:94–102

Millero FJ, Woosley R, Ditrolio B, Waters J (2009a) Effects of ocean acidification on the speciation of metals in seawater. Oceanogr Mar Biol Annu Rev 22:72–85

Millero F, Woosley R, DiTrolio B, Waters J (2009b) Effect of ocean acidification on the speciation of metals in seawater. Oceanography 22(4):72–85

Monari M, Matozzo V, Foschi J, Cattani O, Serrazanetti GP, Marin MG (2007) Effects of high temperatures on functional responses of haemocytes in the clam *Chamelea gallina*. Fish Shellfish Immunol 22:98–114

Mouthon J, Daufesne M (2006) Effects of the 2003 heatwave and climate warning on mollusc communities of the Saone: a large lowland river and of its two main tributaries (France). Glob Chang Biol 12:441–449

Nicolas JL, Comps M, Cochennec N (1992) Herpes-like virus infecting Pacific oyster larvae, *Crassostrea gigas*. Bull Eur Assoc Fish Pathol 12(1):11–13

Nicolas J, Corre S, Gautheir G, Robert R, Ansquer D (1996) Bacterial problems associated with scallop *Pecten maximus* larval culture. Dis Aquat Organ 27:67–76

O'Donnell M, George MN, Carrington E (2013) Mussel byssus attachment weakened by ocean acidification. Nat Clim Chang 3:587–590

Ortega L, Celentano E, Delgado E, Defeo O (2016) Climate change influences on abundance, individual size and body abnormalities in a sandy beach clam. Mar Ecol Prog Ser 545:203–213

Park KI, Yang HS, Kang HS, Cho M, Park KJ, Choi KS (2010) Isolation and identification of *Perkinsus olseni* from feces and marine sediment using immunological and molecular techniques. J Invertebr Pathol 105:261–269

Paynter KT, Politano V, Lane HA, Allen SM, Meritt D (2010) Growth rates and prevalence of *Perkinsus marinus* in restored oyster populations in Maryland. J Shellfish Res 29:309–317

Peeler JE, Reese RA, Cheslett DL, Geoghegan F, Power A, Trush MA (2012) Investigation of mortality in Pacific oysters associated with Ostreid herpesvirus-1 μVar in the Republic of Ireland in 2009. Prev Vet Med 105:136–143

Queiroga FR, Vianna RT, Vieira CB, Farias ND, Da Silva PM (2015) Parasites infecting the cultured oyster *Crassostrea gasar* (Adanson, 1757) in Northeast Brazil. Parasitology 142:756–766

Ramakritinan CM, Chandurvelan R, Kumaraguru AK (2012) Acute toxicity of metals: Cu, Pb, Cd, Hg and Zn on marine molluscs, *Cerithidea cingulate* G., and *Modiolus philippinarum* H. Indian J Geomarine Sci 41(2):141–145

Remacha-Trivino A, Borsay-Horowitz D, Dungan C, Gual-Arnau X, Gomez-Leon J, Villamil L, Gomez-Chiarri M (2008) Numerical quantification of *Perkinsus marinus* in the American oyster *Crassostrea virginica* (Gmelin, 1791) (Mollusca: Bivalvia) by modern stereology. J Parasitol 94:125–136

Renault T, Cochennec N, Le Deuff RM, Chollet B (1994a) Herpes-like virus infecting Japanese oyster (*Crassostrea gigas*) spat. Bull Eur Assoc Fish Pathol 14:64–66

Renault T, Le Deuff RM, Cochennec N, Maffart P (1994b) Herpesviruses associated with mortalities among Pacific oyster, *Crassostrea gigas*, in France – comparative study. Rev Med Vet 145:735–742

Renault T, Chollet B, Cochennec N, Gerard A (2002) Shell disease in eastern oysters, *Crassostrea virginica*, reared in France. J Invertebr Pathol 79:1–6

Renault T, Moreau P, Faury N, Pepin JF, Segarra A, Webb S (2012) Analysis of clinical ostreid herpesvirus 1 (Malacoherpesviridae) specimens by sequencing amplified fragments from three virus genome areas. J Virol 86:5942–5947

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong CS, Wallace DWR, Tilbrook B, Millero FJ, Peng TH, Kozyr A, Ono T, Rios AF (2004) The oceanic sink for anthropogenic CO_2. Science 305:367–371

Saulnier D, De Decker S, Haffner P, Cobret L, Robert M, Garcia C (2010) A large scale epidemiological study to identify bacteria pathogenic to pacific oyster *Crassostrea gigas* and correlation between virulence and metalloprotease-like activity. Microb Ecol 59:787–798

Segarra A, Pepin JF, Arzul I, Morga B, Faury N, Renault T (2010) Detection and description of a particular Ostreid herpesvirus 1 genotype associated with massive mortality outbreaks of Pacific oysters, *Crassostrea gigas*. Virus Res 153:92–95

Sellner KG, Shumway SE, Luckenbach MW, Cucci TL (1995) The effects of dinoflagellate blooms on the oyster *Crassostrea virginica* in Chesapeake Bay. In: Lassus P, Arzul G, Erard-LeDen E, Gentien P, Marcaillou-LeBaut C (eds) Harmful algal blooms. Lavoisier, Paris, pp 505–512

Shi W, Zhao X, Han Y, Che Z, Chai X, Liu G (2016) Ocean acidification increases cadmium accumulation in marine bivalves: a potential threat to seafood safety. Sci Rep 6:20197

Shirayama Y, Thornton H (2005) Effect of increased atmospheric CO_2 on shallow water marine benthos. J Geophys Res Oceans 110:C09S08

Smolowitz R (2013) A review of current state of knowledge concerning *Perkinsus Marinus* effects on *Crassostrea virginica* (Gmelin) (the eastern oyster). Vet Pathol 50:404–411

Soletchnik P, Ropert M, Mazurié J, Fleury PG, Le Coz F (2007) Relationships between oyster mortality patterns and environmental data from monitoring databases along the coasts of France. Aquaculture 271(1–4):384–400

Soniat TM (1996) Epizootiology of *Perkinsus marinus* disease of eastern oysters in the Gulf of Mexico. J Shellfish Res 15:35–43

Talmage SC, Gobler CJ (2011) Effects of elevated temperature and carbon dioxide on the growth and survival of larvae and juveniles of three species of Northwest Atlantic bivalves. PLoS One 6(10):e26941

Tan KS, Ransangan J (2015) Factors influencing the toxicity, detoxification and biotransformation of paralytic shellfish toxins. In: Whitacre DM (ed) Reviews of environmental contamination and toxicology volume 235. Springer International Publishing Switzerland, Basel, pp 1–25

Tan KS, Ransangan J (2016a) Feeding behaviour of green mussels, *Perna viridis* in Marudu Bay, Malaysia. Aquacult Res 48(3):1216–1231

Tan KS, Ransangan J (2016b) Feasibility of green mussel, *Perna viridis* farming in Marudu Bay, Malaysia. Aquac Rep 4:130–135

Tan KS, Ransangan J (2016c) High mortality and poor growth of green mussels, *Perna viridis*, in high chlorophyll-a environment. Ocean Sci J 51(1):43–57

Tan KS, Ransangan J (2016d) Effects of environmental conditions and nutrients on the occurrence and distribution of potentially harmful phytoplankton in mesotrophic water. Sains Malaysiana 45(6):865–877

Tan KS, Ransangan J (2017) Effects of nutrients and zooplankton on the phytoplankton community structure in Marudu Bay. Estuar Coast Shelf Sci 194:16–29

Thomas MK, Kremer CT, Klausmeier CA, Litchman E (2012) A global pattern of thermal adaptation in marine phytoplankton. Science 338:1085–1088

Tracey GA (1988) Feeding reduction, reproductive failure, and mortality in *Mytilus edulis* during the 1985 "brown tide" in Narragansett Bay, Rhode Island. Mar Ecol Prog Ser 50:73–81

Tubiash HS, Chanley PE, Leifson E (1965) Bacillary necrosis, a disease of larval and juvenile bivalve mollusks. I. Etiology and epizootiology. J Bacteriol 90:1036–1044

Vohmann A, Borcherding J, Kureek A (2009) Strong body mass decrease of the invasive clam Corbicula fluminea during summer. Biol Invasions 12:53–64

Wazniak CE, Glibert PM (2004) Potential impacts of brown tide, *Aureococcus anophagefferens*, on juvenile hard clams, *Mercenaria mercenaria*, in the coastal bays of Maryland, USA. Harmful Algae 3:321–329

Wendling CC, Wegner KM (2013) Relative contribution of reproductive investment, thermal stress and *Vibrio* infection to summer mortality phenomena in Pacific oysters. Aquaculture 412–413:88–96

Werner S, Rothhauot KO (2008) Mass mortality of the invasive bivalve *Corbicula fluminea* induced by a severe low-water event and associated low water temperatures. Hydrobiologia 613:143–150

Whyte C, Swan S, Davidson K (2014) Changing wind patterns linked to unusually high *Dinophysis* blooms around the Shetland Islands, Scotland. Harmful Algae 39:365–373

Wikfors GH (2005) A review and new analysis of trophic interactions between *Prorocentrum minimum* and clams, scallops, and oysters. Harmful Algae 4:585–592

Wikfors GH, Smolowitz RM (1993) Detrimental effects of a *Prorocentrum* isolate upon hard clams and bay scallops in laboratory feeding studies. In: Smayda TJ, Shimizu Y (eds) Toxic phytoplankton blooms in the sea. Elsevier, New York, pp 447–452

Wootton EC, Dyrynda EA, Ratcliffe NA (2003) Bivalve immunity: comparisons between the marine mussel (*Mytilus edulis*), the edible cockle (*Cerastoderma edule*) and the razor-shell (*Ensis siliqua*). Fish Shellfish Immunol 15:195–210

Yamamoto C, Tanaka Y (1990) Two species of harmful red tide plankton increased in Fukuoka Bay. Bull Fukuoka Fisheries Exp Station 16:43–44

Yurimoto T, Kassim FM, Fuseya R, Man A (2014) Mass mortality event of the blood cockle, *Anadara granosa*, in aquaculture ground along Selangor coast, Peninsular Malaysia. Int Aquat Res 6:177–186

Zha S, Liu S, Su W, Shi W, Xiao G, Yan M, Liu G (2017) Laboratory simulation reveals significantly impacts of ocean acidification on microbial community composition and host-pathogen interactions between the blood clam and *Vibrio harveyi*. Fish Shellfish Immunol 71:393–398

Review of the Effects of Perinatal Exposure to Endocrine-Disrupting Chemicals in Animals and Humans

William Nelson, Ying-Xiong Wang, Gloria Sakwari, and Yu-Bin Ding

Contents

Abbreviations

AGD	Anogenital distance
BBP	Benzyl butyl phthalate
BPA	Bisphenol A
DBP	Dibutyl phthalate
DDE	4,4′-Dichlorodiphenyldichloroethylene

W. Nelson · Y.-X. Wang · Y.-B. Ding (✉)
Joint International Research Laboratory of Reproductive and Development, Department of Reproductive Biology, School of Public Health, Chongqing Medical University, Chongqing, People's Republic of China

G. Sakwari
Department of Environmental and Occupational Health, School of Public Health and Social Sciences, Muhimbili University of Health and Allied Sciences, Dar es salaam, Tanzania

P. de Voogt (ed.), *Reviews of Environmental Contamination and Toxicology, Volume 251*, Reviews of Environmental Contamination and Toxicology Volume 251,
https://doi.org/10.1007/398_2019_30

DDT	4,4′-Dichlorodiphenyltrichloroethane
DEHP	Di (2-ethyhexyl) phthalate
EDCs	Endocrine-disrupting chemicals
ER	Estrogen receptor
FV	Fenvalerate
GD	Gestational day
HCB	Hexachlorobenzene
IQ	Intelligent quotient
MCNP	Monocarboxyisononyl phthalate
NOAELs	No observed adverse effect levels
NP	4-Nonylphenol
NTD	Neural tube defect
OP	4-Tert-octylphenol
PBB	Polybrominated biphenyl
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PFASs	Perfluoroalkyl substances
PND	Postnatal day
SD	Sprague-Dawley
TBTCl	Tributyltin chloride
TCDD	Tetrachlorodibenzo-*p*-dioxin
TPTCl	Triphenyltin chloride
VZ	Vinclozolin

1 Introduction

Endocrine-disrupting chemicals (EDCs) are exogenous substances that interfere with the synthesis, metabolism, or action of hormones (U.S. Environmental Protection Agency 1997). They are put into two groups: persistent and nonpersistent chemicals. Persistent chemicals persist in the environment and the human body and undergo biomagnification in the food chain. They can persist for a short duration or for decades (Jiang et al. 2014). Nonpersistent chemicals are short-lived in the environment and are rapidly metabolized in and excreted from the human body. Both categories of EDCs are ubiquitously abundant in the environment. Human beings are exposed to EDCs through air and water pollution, contamination of the food chain, and consumer products (Gore et al. 2014). Human exposures have been associated with several noncommunicable diseases that have been increasing in recent decades (U.S. Environmental Protection Agency 2013).

Effects of perinatal exposure to EDCs attracted global attention after the early 1980s. It was observed that pregnant mothers who were treated with diethylstilbestrol (DES) – a synthetic estrogen – had their sons presenting with an increased risk of hypospadias, cryptorchidism, decreased sperm count (Stillman 1982), and an increased risk of developing testicular cancer (Arai et al. 1983). Analysis of pregnancy data from the National Health and Nutrition Examination Survey (NHANES)

showed that pregnant women are ubiquitously exposed to multiple chemicals during a sensitive period of fetal development. At least 43 different environmental chemicals were detected in the bloodstream of pregnant women (Woodruff et al. 2011). Fetuses are exposed to these chemicals in utero when they cross the placental barrier by diffusion either transcellularly through the syncytiotrophoblast layer or paracellularly through water channels incorporated into the membrane (Griffiths and Campbell 2015). Exposure continues after birth through breastfeeding (Agatonovic-Kustrin et al. 2002). Studies report that lactational transfer of EDCs in mammals during nursing is greater than that of placental transfer (Desforges et al. 2012; Shin et al. 2017). For example, brominated flame-retardant (2-ethylhexyl-2,3,4,5-tetrabromobenzoate) body burdens resulting from the lactational transfer are approximately 200- to 300-fold higher than those resulting from the placental transfer (Phillips et al. 2016).

Large volumes of evidence have been generated on the adverse health effects of perinatal exposure to EDCs, raising the need for a review to summarize the existing knowledge and identify gaps in knowledge and recommend priorities for future research. This review summarizes the research evidence from animal experimental and human epidemiological studies on the impact of in utero and lactational exposure to selected EDCs on the health status of the first generation (F1) and subsequent generations of the exposed offspring. EDCs addressed in this review are grouped into two: nonpersistent and persistent chemicals. Nonpersistent chemicals include phenols and phthalates. The phenols comprise bisphenol A (BPA), 4-nonylphenol (NP), and 4-tert-octylphenol (OP). Persistent chemicals include organochlorides [polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), 4,4′-dichlorodiphenyltrichloroethane (DDT), and 4,4′-dichlorodiphenyldichloroethylene (DDE)], polybrominated compounds [polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB)], dioxins and dioxin-like compounds, fenvalerate (FV), tributyltin chloride (TBTCl), perfluoroalkyl substances (PFASs), triphenyltin chloride (TPTCl), and vinclozolin (VZ).

2 Methods

2.1 Search Strategy

Descriptive reviews of animal experimental and human epidemiological studies were performed by conducting an electronic search of literature published between 1 January 2000 and 29 October 2018 in MEDLINE on the PubMed platform, Web of Science (webofknowledge.com), and Toxline (National Library of Medicine, USA). We developed and performed the bibliographic searches using subject thesaurus vocabulary (MeSH), keywords, and text words for each of the search concepts. The search strategy employed each of the following terms: developmental, maternal, perinatal, gestational, in utero, early life, intrauterine, fetal, exposure, endocrine-disrupting chemicals, endocrine disruptors, specific chemical names, health effects, and offspring. These terms were searched using the Boolean operators: “OR”

and "AND." Retrievals were limited to studies in English. Search results were downloaded to Mendeley software (Elsevier) to merge references and remove duplicates.

2.2 Screening and Eligibility

Article screening and eligibility followed PRISMA 2009 Flow Diagram (Moher et al. 2009). Each level of review (Fig. 1) was completed in duplicates by authors W.N.M, G.S, and Y.D. At the title and abstract screening level, we included all rodents (rat and mice) experimental and human epidemiological studies with parental exposure to known EDCs and which considered offspring health as an outcome.

Endocrine-disrupting chemicals were limited to persistent (PCBs, PBDEs, organochlorides, dioxins, and dioxin-like compounds, fenvalerate, TBT, PFASs,

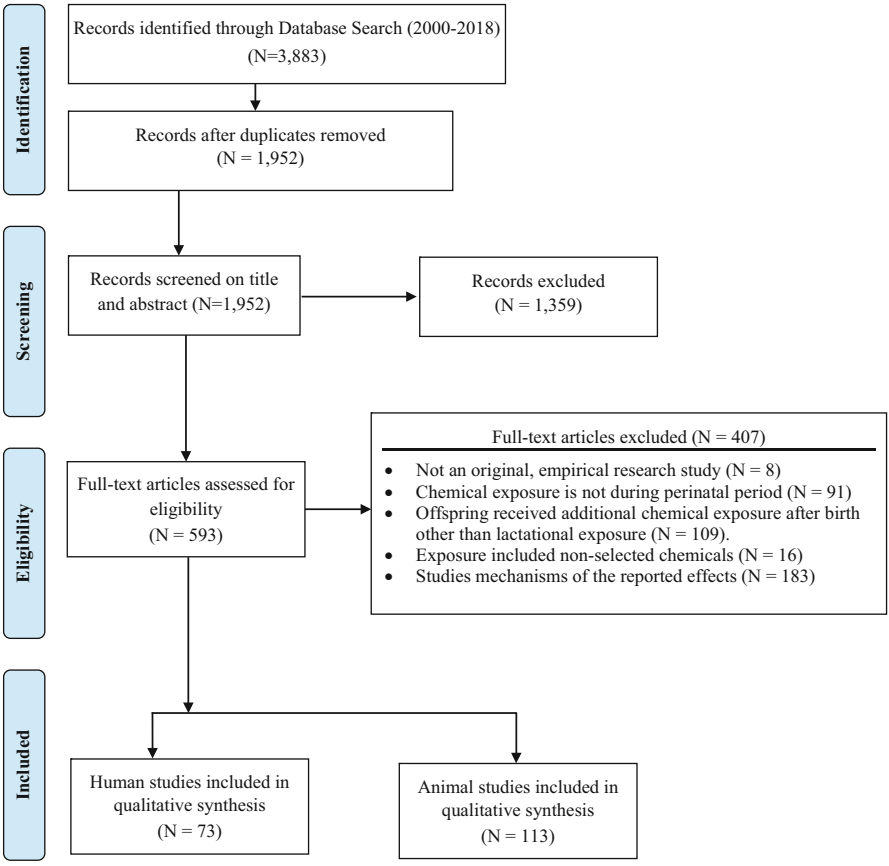

Fig. 1 PRISMA flow chart

TPT, and vinclozolin) and nonpersistent (BPA, OP, phthalates, and NP) pollutants. In animal experimental studies, the perinatal period was considered as chemical exposure during gestation and lactation or gestation period alone, but not lactational period alone. In addition, animals were restricted to rodents, particularly mice and rats. Also, human epidemiological studies, which quantified chemical exposures from maternal biological media, during gestation and/or lactation or at birth, were used for this review.

We obtained full-text articles of all titles that satisfied the inclusion criteria. At the second level of screening, we included only original empirical research studies that considered offspring health as an outcome and which examined exposure to selected chemicals during the perinatal period.

2.3 Risk of Bias and Quality Assessment of Epidemiological Studies

We adopted the Newcastle-Ottawa Scale (NOS) for cohort and case-control studies (Wells et al. 2013) to assess the risk of bias and quality of studies in three domains: participant selection/exposure, comparability of groups, and outcome assessment (Table 1). Each assessment criterion was awarded a score of 1 or 0, making a maximum of 9 quality score points. Specifically, good-quality studies were identified as those awarded 5–6 points in the selection/exposure domain, 1 point in the comparability domain, and 2 points in the outcome domain. Fair studies were indicated by 3–4 points for selection/exposure, 1 point for comparability, and 2 points for the outcome, whereas poor-quality studies scored <3 for selection/exposure or 0 for comparability OR ≤ 1 for the outcome.

3 Results

The search resulted in a total 3,883 articles. And after we had removed duplicates, only 1,952 potentially relevant journal articles remained. Of these remaining articles, 1,359 were excluded at the title and abstract screening level, leaving only 593 full-text articles which were later assessed for eligibility. One hundred thirteen rodent and 73 human studies met the inclusion criteria and hence were used for this study (Fig. 1).

Table 1 Assessment of risk of bias and study quality

Assessment scale items	Criteria for higher-quality selection	Score
Selection		
Representativeness of the exposed cohort	Truly or somewhat representative of the exposed population	1 or 0
Adequacy of exposure measure	A measure of maternal chemical concentration in urine, blood, breast milk, or cord blood exposure level	1 or 0
	A measure of health outcomes in the offspring relative to maternal chemical concentrations. A statistical association between chemical concentrations in maternal biological media and the offspring's health outcome established	1 or 0
Study design	Cohort or cross-sectional studies	1 or 0
Exposed population	Mother. No additional exposure in the offspring	1 or 0
Outcome population	Exposed male and/or female offspring, i.e., fetus, newborn, infant, or young children exposed in utero and/or during lactation to the chemical	1 or 0
Comparability		
Comparability of cohorts on the basis of the design or analysis	Outcome measure confounded by one of the factors like smoking status, maternal age, birth weight, maternal BMI, gestational age, breastfeeding duration, etc.	1 or 0
Outcome		
Assessment of outcome	A measure of individual (maternal) chemical concentration during pregnancy, at delivery or breastfeeding	1 or 0
	Measurement of individual (child) health outcome done by trained practitioner blinded of the mother's exposure status	1 or 0
Total quality score		

3.1 Description of Studies

Characteristics of the 113 rodent and 73 human studies are summarized in Tables 2 and 3, respectively. Animal studies have focused much on the health effects of perinatal exposure to nonpersistent chemicals (phenols and phthalates) (59%; $n = 67$). Many nonpersistent chemicals' studies focused on offspring's reproductive health (46%; $n = 52$). Eighteen studies (15.9%) covered offspring effects in more than one generation (multigenerational); seven covered two generations; eight covered three generations, and three covered four generations.

On the other hand, 81% ($n = 59$) of the human studies addressed the effects of persistent chemicals, and 41% ($n = 30$) focused on offspring growth and development (Table 4). By using NOS scores, we found that 43 of these articles were of good quality, while the other 30 were of fair quality. All human studies on nonpersistent chemicals (phenols and phthalates) used maternal urine samples to quantify

Table 2 Characteristics of animal studies

Article ID	Chemical	Species	Dosing	Exposure period	Route	Effect assessment period
Acevedo (2013)	BPA	SD rats	0 or 250 μg BPA/kg BW/day	GD18	Osmotic pumps	72 h
Ahmad (2014)	DBP and BBP	Albino rats	DBP-2, 10, and 50 mg/kg, BBP-4, 20, and 100 mg/kg	GD14–birth	Oral	At birth
Alonso-Magdalena (2010)	BPA	OF-1 mice	10 or 100 μg/kg/day	GD9–16	s.c. inj.	At 4 and 6 months
Altamirano (2015)[a]	BPA	Wistar rats	0.6 μg or 52 μg BPA/kg/day	GD9–PND21	Drinking water	GD21, LD 2, and 10
Angle (2013)	BPA	CD-1 mice	5, 50, 500, 5,000, or 50,000 μg/kg-BW/day	GD9–18	Micropipette	From birth to 5 months
Anh (2013)	TCDD	Wistar rats	1.0 μg/kg	GD15	Oral gavage	8–9 weeks
Anway (2006)[b]	VN	SD and fisher rats	100 mg/kg/day	E8–E14 year	i.p. injection	PND60
Aragon et al. (2008)	TCDD	C57BL/6N mice	6 μg/kg	GD14.5	Oral gavage	At 3.5 months
Arambula et al. (2017)	BPA	SD rats	2.5, 25, or 2,500 mg/kg-BW/day	GD6–PND21	Oral gavage	At PND28
Bansal (2017)[a]	BPA	C57BL/6 mice	10 μg/kg/day or 10 mg/kg/day	14 days pre-mating–PND21	Diet	16–21 weeks
Barakat (2018)	DEHP	CD-1 mice	200 μg, 500 mg, or 750 mg/kg/day	GD11–birth	Micropipette	16–22 months
Barakat (2017)	DEHP	CD-1 mice	20, 200, 500, or 750 μg/kg/day	GD11–birth	Micropipette	PND8–23 months
Bell et al. (2007)	TCDD	Wistar rats	50, 200, or 1,000 ng/kg BW	GD15	Oral gavage	PND70 and 120
Bell et al. (2018)	PCB	SD rats	20 μg/kg	E1–birth	Diet	1 day old
Boberg (2011)	DINP	Wistar rats	300, 600, 750, or 900 mg/kg-BW/day	GD7–PND17	Oral gavage	GD21, PND22, and 90
Bodin (2014)	BPA	NOD/ShiLtJ mice	30, 300, or 3,000 μg/kg/day	Mating–PND21	Drinking water	7–28 weeks

(continued)

Table 2 (continued)

Article ID	Chemical	Species	Dosing	Exposure period	Route	Effect assessment period
Boudalia (2014)[a]	BPA	Wistar rats	5 µg/kg-BW	GD1–PND21	Oral	PND21–100
Bowers (2004)	Mixture, PCBs, and OCPs	SD rats	0.013, 0.13, 1.3, or 13 mg/kg (mix) or 15 mg/kg of PCB	GD1–PND23	Diet	PND6–14 and PNDs 35, 75, and 350
Braniste (2010)	BPA	Wistar rats	5 mg/kg/day	GD15–PND21	Oral gavage	10 weeks postpartum
Bushnell et al. (2002)	PCBs (A1254)	Long-Evans rats	0, 1.0, or 6.0 mg/kg/day	GD6–PND21	Oral gavage	PND17, 28, 43, and 65
Cabaton (2013)	BPA	CD-1 mice	0.025, 0.25, or 25 µg BPA/kg-BW/day	GD8–PND16	Osmotic pumps	PND2 and PND21
Cagampang (2012)	BPA	MF-1 mice	100 mg/kg-BW	GD11–19	s.c. injection	
Carbone (2012)	DEHP	Wistar rats	3 and 30 mg/kg BW/day	GD1–weaning	Drinking water	15 days of age
Chamorro-Garcia (2012)[b]	TBT	C57BL/6J mice	DMSO or 50 nM TBT + diet challenge	7 days prior mating–PND21	Drinking water	33 weeks of age
Chamorro-Garcia (2013)[c]	TBT	C57BL/6J mice	DMSO or 5.42, 54.2, or 542 nM TBT	7 days prior mating–Delivery	Drinking water	8 weeks of age
Chang (2014)	NP	SD rats	400 µg/kg/day (preg.) and 800 µg/kg/day (lact.)	GD1–PND21	Drinking water	13–14 weeks of age
Colciago (2009)	PCBs	SD rats	10 mg/kg daily	GD15–PND21	s.c. inj.	PND60
Dai (2015)	DEHP	ICR mice	10, 50, and 200 mg/kg/day	GD7–PND21	Orally	PND42–84
DeBartolo et al. (2016)	BBP	SD rats	10.0 µg/mL (5–7 days exp. during preg)	E 14–PND23	Diet	PND23 or 65
Dessì-Fulgheri et al. (2002)	BPA	SD rats	40 µg/kg/day (L) or 400 µg/kg/day (H)	10 days pre-mating–PND21 (L) and GD14 (±1 day)–PND6 (H)	Micropipette	PNDs 35, 45, and 55
Doyle et al. (2013)[b]	DEHP	CD1 mice	F0–500 mg/kg BW/day	E7–E14	Oral gavage	PND30 and 120 (F1–F4); PND360 (F4)

Dunder et al. (2018)	BPA	Fischer 344 rat	0.5 or 50 μg/kg BW/day	GD3.5–PND22	Drinking water	5-week-old male and 52-week-old female
Ema and Miyawaki (2002)	BBP	Wistar rats	250, 500, or 1,000 mg/kg	GD15–17	Gastric intubation	PND21
Ema and Miyawaki (2001)	MBuP	Wistar rats	250, 500, or 750 mg/kg	GD15–18	Gastric intubation	PND22
Endo et al. (2012)	TCDD	C57BL/6 mice	0.6 or 3.0 mg/kg	GD12.5	Orally	PND180
Fenton et al. (2002)	TCDD	Long-Evans rats	1 μg TCDD/kg-BW	GD15	Oral gavage	PND4, 33, 37, 45, 68, and 110
Fielden et al. (2001)	PCB (A1242)	C57BL6 mice	10, 25, 50, and 100 mg/kg-BW	2 weeks prior to mating–PND21	Oral gavage	16 and 45 weeks of age
Galyon et al. (2017)	BPA	SD rats	239 ± 8 μ mg/day/BW GD1–birth and 466 ± 33 μg/day/BW PND1–21	2 weeks prior to GD–PND21	Drinking water	At 6 and 24 weeks
Gillette et al. (2017)	PCB (A1221)	SD rats	0.5 mg/kg or 1.0 mg/kg, hereafter 1.0 and 0.5	Twice; GD16 and 18	i.p. injection	AGD PND1–14. Sexual maturity – PND28 and 35
Gu et al. (2016)	DEHP	C57BL/6J mice	0.05 or 500 mg/kg/day	GD1–19	Oral gavage	9 weeks of age
Guerra et al. (2010)	DBP	Wistar rats	100 mg/kg/day	GD12–PND21	Oral gavage	PND60 and 75
Haavisto et al. (2006)	TCDD	SD rats	0.04, 0.2, or 1.0 μg/kg	GD13	Oral gavage	PND14
Hamm et al. (2000)	TCDD	Long-Evans rats	1.0 μg/kg	GD15	Oral gavage	PND15, 25, 32, 49, 63, and 120
Hass et al. (2016)	BPA	Wistar rats	25 μg, 250 μg, 5 mg or 50 mg/kg BW/day	GD7–PND22	Oral gavage	PND105–116- and 9-month male/14-month female
Hojo et al. (2008)	TCCD	Long-Evans Hooded rats	TCDD (50, 200, or 800 ng/kg); PCB126 (500, 2,000, or 8,000 ng/kg)	GD15	Oral gavage	From 11 weeks of age for 30 days

(continued)

Table 2 (continued)

Article ID	Chemical	Species	Dosing	Exposure period	Route	Effect assessment period
Honma et al. (2002)	BPA	ICR/Jcl mice	2 or 20 μg/kg	GD11–17	s.c. injection	PNDs 22 and 60
Ichihara et al. (2003)	BPA	F344 rats	0.05, 7.5, 30, 120 mg/kg/day	GD1–PND21	Oral gavage	At 65 weeks of age
Ikeda et al. (2005)	TCCD	Holtzman rats	800 and 200 ng/kg	GD15	Oral gavage	PND2
Jang et al. (2012)[a]	BPA	C57BL/6 mice	0.1, 1, or 10 mg/kg	GD6–17	i.p. injection	At 6 weeks
Jašarević et al. (2013)	BPA	Outbred deer mice	50 mg, 5 mg, 50 μg/kg feed weight	2 weeks prior to GD; GD1–PND21	Diet	≈60 days of age
Jin et al. (2008)	TCDD	C57BL/6 mice	1 μg/kg	GD15	i.p. injection	Immature (PND30) and mature (PND60)
Jones et al. (2011)	BPA	Long-Evans rats	5, 50, 500 μg/kg-BW/day, or 5 mg/kg-BW/day	GD7–PND14	Oral gavage	90–120 days of age
Kakeyama et al. (2014)	TCDD	Long-Evans rats	200 or 800 ng/kg	GD15	Oral gavage	Adulthood
Kakeyama et al. (2008)	TCDD	Long-Evans hooded rats	200 or 800 ng/kg-BW	GD15	Oral gavage	PND25
Kawai et al. (2003)	BPA	CD-1 mice	2 ng/g or 20 ng/g	GD11–17	Micropipette	8, 12, and 16 weeks of age
Kaya et al. (2002)	PCBs	Long-Evans hooded rats	0.5, 2, or 4 mg/kg-BW/day	50 days pre-mating–PND0	Diet	PND21 and PND110
Kim et al. (2010)	DBP	SD rats	250, 500, or 700 mg/kg/day	GD10–19	Oral gavage	Male off sacrificed at 31 days of age
Kobayashi et al. (2002)	BPA	SD rats	4 or 40 mg/kg-BW/day	GD6–PND20	Oral gavage	1, 3, and 9 weeks of age

Kobayashi et al. (2009)	PCB 153	SD rats	1 or 4 mg/kg/day	GD10–16	Oral gavage	1, 3, or 9 weeks of age
Kodavanti et al. (2010)	PBDEs (DE-71)	Long-Evans rats	1.7, 10.2, or 30.6 mg/kg/day	GD6–PND21	Oral gavage	PND7 and PND30 onward
Kuriyama and Chahoud (2004)	PCB 118	SD rats	375 mg of PCB 118/kg-BW	GD6	Oral gavage	PND70–74
Kwon et al. (2000)	BPA	SD rats	3.2, 32, or 320 mg/kg/day	GD11–PND20	Oral gavage	PND27 onward
La et al. (2014)	DDT	C57BL/6J mice	1.7 mg DDT/kg-BW	11.5–PND5		Several ages up to 9 months
Lee et al. (2016)	DEHP	C57BL/6 mice	30 mg/kg-BW	4 weeks pre-mating–PND21	Oral gavage	8 weeks old
Lejonklou et al. (2016)	BPA	Wistar rats	25, 250, 5,000, or 50,000 μg BPA/kg-BW/day	GD7–PND22	Oral gavage	3 months old
Li et al. (2014)	BPA	SD rats	40 μg/kg-BW	GD0–PND21	Oral gavage	9 weeks and 20 weeks old F2
Li et al. (2013a)	DEHP	SD rats	500, 750, or 1,000 mg/kg-BW/day	GD12–19	Gastric intubation	PND30 and PND60
Lin et al. (2011)	DEHP	Wistar rats	1.25 and 6.25 mg/kg/day	GD0–PND21	Oral gavage	27 weeks old
Lind et al. (2017)	BPA	Fischer 344 rat	0.5 or 50 μg/kg BW/day	GD3.5–PND22	Drinking water	5 weeks old
Ma et al. (2017)	DBP	SD rats	50, 250, or 500 mg/kg/day	GD12.5–21.5	Oral gavage	PND9, 21, 35, and 90
Makita et al. (2004)	DDE and TBT	Wistar rats	126 ppm (DDE) and 25 ppm TBT	GD1–PND22	Diet	PND4; PND35–43
Mandrup et al. (2016)	BPA	Wistar rats	0.025, 0.25, 5, and 50 mg/kg-BW/day	GD7–PND22	Oral gavage	At 22, 100, and 400 days old
Martinez-Arguelles et al. (2013)	DEHP	SD rats	300 mg DEHP/kg/day	GD14–birth	Oral gavage	PND60 and 200
Mennigen et al. (2018)[c]	PCB (A1221)	SD rats	1 mg/kg	E 16 and E18	i.p. and s.c. injections	PND58–63

(continued)

Table 2 (continued)

Article ID	Chemical	Species	Dosing	Exposure period	Route	Effect assessment period
La Merrill et al. (2010)	TCDD + fat diet	FVB/NJ mice	1 μg/kg TCDD	GD12.5	Orally	PND50 and 83
Moon et al. (2007)	NP	Long-Evans rats	10 or 100 mg/kg	GD15–19	Oral gavage	PND22
Moore et al. (2001)	DEHP	SD rats	375, 750, or 1,500 mg/kg/day	GD3–PND21	Orally	PND24–63
Mylchreest et al. (2000)	DBP	CD rats	0.5, 5, 50, or 100, or 500 mg/kg/day	GD12–21	Oral gavage	PND1 and 14
Nassr et al. (2010)	FV	Wistar rats	40 or 80 mg/kg	GD12–PND21	Oral gavage	PND40, 60, and 90
Nguyen et al. (2013)	TCDD	Wistar rats	1.0 μg/kg	GD15	Oral gavage	8–9, 11–12, and 19 weeks of age
Nilsson et al. (2008)[c]	VZ	SD rats	100 mg/kg/day	E8–E14	i.p. injections	
Nishijo et al. (2007)	TCDD	Wistar rats	0.1 μg/kg	GD9–19	Gastric intubation	PND4–14 and PND31–44
Ohsako et al. (2002)	TCDD	SD rats	1 μg TCDD/kg body	GD15 or 18	Oral dose	PND70
Orito et al. (2007)	PCB 126	SD rats	30 μg/kg	GD15	Orally	4–5 weeks old
Pocar et al. (2017)[c]	DEHP	CD-1mice	0.05, 5 mg/kg/day	GD0.5–PND21	Diet	PND42
Ryan et al. (2010)	BPA	Long-Evans rat	2, 20, and 200 mg/kg/day	GD7–PND18	Oral gavage	PND2–23
Salian et al. (2009)[c]	BPA	Holtzman rats	1.2 and 2.4 μg/kg-BW/day	GD12–PND21	Oral gavage	PND75 and PND125
Sanabria et al. (2016)[c]	TCDD	Wistar rats	0.1, 0.5, and 1.0 μg of TCDD	GD15	Oral gavage	Adult

Schneider et al. (2008)[c]	VZ	Wistar rats	4 or 100 mg/kg-BW/day	GD6–15	Oral gavage	At age 127–134 days
Schönfelder et al. (2002)	BPA	SD rats	0.1 or 50 mg/kg/day	GD6–21	Oral gavage	4 months of age
Shono et al. (2004)	VZ	Wistar King A rats	200 mg/kg/day	GD15–18	Oral gavage	AGD at birth, PND10 and 60
Si et al. (2012a)	TBTCl	Chinese Kun Ming mice	1, 10, or 100 μg TBTCl/ kg-BW/day	GD6–PND21	Oral gavage	PND21 (VO); PNDs 49 and 152
Si et al. (2012b)	TBTCl	Chinese Kun Ming mice	1, 10, or 100 μg TBTCl/ kg-BW/day	GD6–PND22	Oral gavage	PNDs 3, 5, 7, and 9
Song et al. (2014)	BPA	SD rats	1 and 10 μg/mL BPA	GD6–PND21	Drinking water	PND50 and PND100
Spörndly-Nees et al. (2018)	BPA	Fischer 344 rats	0.5 or 50 μg BPA/kg/day	GD3.5–PND22	Drinking water	PND35 and 12 months old
Steinberg et al. (2008)[a]	PCB (A1221)	SD rats	0.1, 1, or 10 mg/kg	E16–18	i.p. injection	≈PND42
Stump et al. (2010)	BPA	SD rats	0.15, 1.5, 75, 750, and 2,250 ppm daily	GD0–PND21	Diet	PNDs 4–72
Sugawara (2006)	PCB (A1254)	C57BL/6Cr mice	6, 18, and 54 mg/kg-BW every 3 days	GD6–PND20	Oral gavage	PNDs 4, 7, 10, 12, 14, and 16
Susiarjo et al. (2015)[a]	BPA	C57BL/6 mice	10 μg/kg/day or 10 mg/kg/day	2 weeks prior to mating–PND21	Diet	PND98–117
Takagi et al. (2004)	BPA and NP	SD rats	BPA, 60, 600, or 3,000 ppm; NP, 60, 600, or 3,000 ppm	GD15–PND10	Diet	PND21 and PNW 8–11
Xu et al. (2015)	DEHP	ICR mice	10, 50, and 200 mg/kg/day	GD7–PND21	Orally	6 weeks old
Andrade et al. (2006)	DEHP	Wistar rats	Low, 0.015, 0.045, 0.135, 0.405, and 1.215, and high, 5, 15, 45, 135, and 405 mg DEHP/kg-BW/day	GD6–PND21	Oral gavage	PND144 ± 7 days
Bruner-Tran and Osteen (2011)[b]	TCDD	C57BL/6 mice	10 μg/kg	GD15.5	Oral gavage	PND21

(continued)

Table 2 (continued)

Article ID	Chemical	Species	Dosing	Exposure period	Route	Effect assessment period
Grote et al. (2007)	TPTCl	Wistar rats	2 or 6 mg TPTCl/kg-BW/day	GD6–PND21	Oral gavage	At birth
Kimura et al. (2006)	NP	ICR mice	1.231, 12.31, or 123.1 mg/kg	GD5–20	s.c. injection	PND42
Kuwahara et al. (2013)	BPA	SD rats	50 or 500 μg/kg/day	GD10–PND14	Orally	7–11 weeks of age
LaRocca et al. (2011)	BPA	C57/Bl6 mice	50 or 1,000 μg/kg BPA	GD10–16	Oral gavage	PND56
Li et al. (2013b)	DBP	SD rats	500 mg/kg-BW	GD6–PND21	Gastric intubation	PND21 and 60
Lilienthal et al. (2006)	PBDE-99	Long-Evans hooded rats	1 or 10 mg/kg-BW/day	GD10–18	s.c. injection	PND30 onward and PND120
Pocock et al. (2002)	OP	Wistar rats	100–250 mg/kg/day	GD0–PND21	Diet	PND0–327
Rubin et al. (2016)	BPA	CD-1 mice	0.25, 2.5, 25, or 250 μg/kg-BW/day	GD8–PND16	Osmotic pumps	Week 3–43
Si et al. (2013)	TBTCl	Chinese Kun Ming mice	1, 10, or 100 μg/kg-BW	GD6–PND21	Oral gavage	PNDs 49 and 152
Yang et al. (2017)	NP	SD rats	200 mg/kg/day	GD6–PND21	Oral gavage	PND60

LD lactation day, *SD* Sprague-Dawley, *s.c.* subcutaneous, *i.p.* intraperitoneal, *PND* postnatal day, *GD* gestational day, *VO* vaginal opening

[a]F1 and F2 generation

[b]F1–F4 generation

[c]F1–F3 generation

Table 3 Characteristics of human studies

Article ID	Chemicals	Country	Study period	No. of mother-children pairs	Maternal biological media	Sampling period	Offspring assessment period
Botton et al. (2016)	Phthalates	France	2003–2006	520	Urine	GW22–29	Mean GW12.6
Braun et al. (2009)	BPA	USA	2013	249	Urine	GW16, 26, and at birth	At 2 years
Brown et al. (2018)	DDT and PCB	Finland	2005–2007	778 cases and 778 controls	Serum	2–4 months of pregnancy	Childhood
Chen et al. (2018)	PFASs	China	2012–2015	687	Cord blood	Birth	At 6, 12, and 24 months
Cupul-Uicab et al. (2013)	Organochlorides	USA	1959–1965	1,683	Serum	Third trimester	7 years
Fleisch et al. (2017)	PFASs	USA	1999–2002	665	Plasma	Median GW9.6	Median 7.7 years
García-Villarino et al. (2018)	POPs (HCB, PBDE, DDD, PCBs)	Spain	2004–2007	43	Blood	Median GW12	At 18 months
Han et al. (2016)	PCBs and DDE	USA	2001–2002	151	Serum	Not indicated	20–50 years
Hansen et al. (2016)	POPs (HCB, PCBs, and DDE)	Denmark	2008–2009	421	Serum	Gest. week 30	At 20 years
Hansen et al. (2014)	POPs (HCB, PCBs, and DDE)	Denmark	2008–2010	654	Serum	Gest. week 31	At 20 years
Jarrell et al. (2002)	HCB	Turkey	1935–1990	42 case-control pairs[a]	Serum	Not indicated	40 years follow-up
Jeddy et al. (2017)	PFASs	Great Britain	1991–1992	432	Serum	Median GW15	At 15 and 38 months
Jeddy et al. (2018)	PFASs	Great Britain	1991–1993	257	Serum	Median GW15	17-year-old daughters

(continued)

Table 3 (continued)

Article ID	Chemicals	Country	Study period	No. of mother-children pairs	Maternal biological media	Sampling period	Offspring assessment period
Jensen et al. (2015)	Phthalates	Denmark	2008–2009	245	Urine	First trimester	3 months after EDD
Jusko et al. (2010)	PCBs	Slovakia	2002–2004	384	Serum	Day reported for delivery and cord blood	At 6 months
Jusko et al. (2012)	DDE and DDT	USA	1959–1965	1,100	Serum	3rd trimester	At 8 months and 7 years
Kalra et al. (2016)	Organochlorides	India	2013–2015	35 case-control pairs[b]	Serum	Within 72 h of delivery	Within 72 h of delivery
Karmaus et al. (2009)	PCBs and DDE	USA	1973–1991	169	Serum	Not indicated	20–50 years
Khanjani and Sim (2007)	DDT and DDE	Australia	1990s	815	Breast milk	6–12 weeks postpartum	Infant
Kim et al. (2018)	BPA, phthalates, PCBs, PBDE, and OCPs	Korea	2011–2012	140	Blood, urine and breast milk[c]	Blood and urine at term, breast milk 30 days after delivery	At 13–24 months
Lauritzen et al. (2018)	POPs	Norway and Sweden	1986–1988	412	Serum	Second trimester (GW17–20)	5-year follow-up
Liew et al. (2018)	PFASs	Denmark	1996–2002	1,592	Serum	Mean GW8.7	At 5 years
Lim et al. (2017)	BPA	Korea	2008–2011; 2014–2015	304	Urine	Second trimester	At 4 years
Longnecker et al. (2004)	PCBs	Denmark	1959–1966	195 cases and 615 controls[d]	Serum	Several[e]	At 8 years
Longnecker et al. (2002)	DDE	USA	1959–1966	585 cases and 522 controls[f]	Serum	Third trimester	Infant
Mcglynn et al. (2009)	PCBs	USA	1959–1965	431 cases and 593 cases[g]	Serum	Third trimester	At 4 months, 1 year, 7 years

Miyashita et al. (2018)	Dioxin-like compounds (DLCs)	Japan	2002–2005	327	Vein blood and cord blood	Second to third trimester, cord blood at delivery	At 3.5 years and 7 years
Miyashita et al. (2011)	Dioxin-like compounds	Japan	2002–2005	364	Maternal blood		At 18 months
Mustieles et al. (2018)	BPA	USA	2005–2016	107	Urine	1 per trimester	Infant
Olesen et al. (2018)	Phthalates	Denmark	2010–2012	518	Urine	Third trimester	At 20–36 months
Shu et al. (2018)	Phthalates	Sweden	2007–2010	1,062	Urine	Median GW10	At 12 months
Starling et al. (2017)	PFAS	USA	2009–2014	652	Serum and cord blood	Mid-pregnancy, cord blood at delivery	Within 3 days of birth
Sun et al. (2018)	BPA	China	2012	982	Urine	GW12–16	At 6 and 12 months
Terrell et al. (2015)	PBB and PCBs	USA	1976–1978	613 offspring from 330 women	Serum	At enrolment	Infant
Vafeiadi et al. (2013)	Dioxins (TCDD) and dioxin-like compounds	Greece and Spain	2007–2008	119 newborn and 239 young boys; 118 newborn and 223 young girls	Blood	At delivery	Range; 1–31 months
Vafeiadi et al. (2015)	POPs (PCBs, HCB, and DDE)	Greece	2007–2008	689	Serum	First trimester	At 4 years of age
Vafeiadi et al. (2016)	BPA	Greece	2007–2008	500	Urine	First trimester	At 2.5 and 4 years
Valvi et al. (2013)	BPA	Spain	2004–2006	402 mothers	Urine (2 samples)	First and third trimester	At 6 and 14 months and 4 years
Vasiliu et al. (2004)	PCBs and DDE	USA	2000	151 female offspring aged 20–50 years	Serum	Not indicated	20 ± 50 years
Vested et al. (2014)	POPs (PCBs and DDE)	Denmark	2008–2009	176	Blood, off-spring – semen and blood samples	GW30	Median 20 years

(continued)

Table 3 (continued)

Article ID	Chemicals	Country	Study period	No. of mother-children pairs	Maternal biological media	Sampling period	Offspring assessment period
Wang et al. (2014)	Organochlorides	China	2010–2013	117 cases and 121 controls[h]	Serum	Placenta	At delivery or termination
Zhu et al. (2018)	Phthalates	China	2014–2014	1,002 mother-infant pairs	Urine	3 days before delivery	Infant
Blanck et al. (2000)	PBBs and PCBs	USA	1997	327 mother-daughter pairs	Serum	After exposure (1976–1979)	At 5–24 years
Braun et al. (2017)	BPA and PBDE	USA	2003–2006; 2012–2014		Urine (BPA) and serum (PBDE)	Urine – GW16 and 26 Serum – GW16	At 1–8 years
Brucker-Davis et al. (2010)	Xenobiotics	France	2002–2005	86 maternal-infant pairs	Cord serum and breast milk	Milk – day 2 and 5 postpartum	Infant
Brucker-Davis et al. (2008)	Xenobiotics	France	2002–2005	164 infant/mother pairs	Cord serum and breast milk	Milk – day 2 and 5 postpartum	Infant
Chang et al. (2013)	Nonylphenol	Taiwan	2010	162 pregnant women	Urine	Each trimester	Neonate
Damgaard et al. (2006)	Organochloride	Finland and Denmark	1997–2001	62 cases and 68 controls[i]	Breast milk	1–3 months postpartum	Neonate
Garced et al. (2012)	DDE	Mexico	2001–2005	253 pregnant women	Serum	Each trimester	From birth to 1 year
Gascon et al. (2013)	POPs (PCB-153, DDE, and HCB)	Spain	2003–2008	1,175	Serum	Between seventh and the 26th week	At 14 months
Givens et al. (2007)	PBB	USA	1997–1998	444 mothers and their 899 infants	Serum	At conception and enrollment	Infant
Gladen et al. (2003)	Organochlorides	Ukraine	1993–1994	197	Breast milk	4 or 5 days after birth	Infant

Hertz-Picciotto et al. (2005)	PCBs	USA	1964–1967	399 mothers	Serum and lipid	Second or third trimester	Infant and 5 years of age
Iszatt et al. (2016)	Dioxin and dioxin-like compounds	Belgium, Norway, and Slovakia	2002–2004	367 for infant growth and 251 for BMI	Cord blood or breast milk	Milk 4–5 days after delivery and 8 consecutive days for 1 month	At birth, 24 h and 7 years
Khanjani and Sim (2007)	PCBs	Australia	1990s	200	Breast milk	6–12 weeks postpartum	Infant
Lauritzen et al. (2017)	PFASs and OCs	Norway and Sweden	1986–1988	143 SGAs and 281 non-SGA	Serum	GW17–20	At GW17
Longnecker et al. (2005)	PCBs	USA	1959–1965	1,034 women with 132 preterm births and 101 SGA births	Serum	Third trimester	Infant
Longnecker et al. (2001)	DDE	USA	1959–1966	2,380 children; 361 preterm and 221 SGA	Serum	Fourth trimester	Infant
Main et al. (2007)	PBDEs	Denmark and Finland	1997–2001	86 placenta-milk pairs	Placenta tissue and Milk	Milk-1 month after birth	Infant
Mendez et al. (2011)	OCs (DDE and HCB)	Spain	2004–2006	657	Serum	First trimester	Infant
Nagayama et al. (2007)	Dioxins, PCBs, and organochlorides	Japan	1994–1996	108	Breast milk	2 and 4 months after delivery	Infants (approx. 10 months)
Pan et al. (2010)	DDT, DDE, and PCBs	USA	2004–2006	210	Breast milk	3 months postpartum	Infant (first 12 months)
Sagiv et al. (2007)	Organochlorines	USA	1993–1998	722	Cord blood	At birth	At 1–2 days
Shoaff et al. (2016)	Phthalate	USA	2003–2006	368	Urine	GW16 and 26	Infant
Smink et al. (2008)	HCB, PCBs, DDT, and DDE	Spain	1997	482	Cord serum	At birth	At age 6.5 years

(continued)

Table 3 (continued)

Article ID	Chemicals	Country	Study period	No. of mother-children pairs	Maternal biological media	Sampling period	Offspring assessment period
Tai et al. (2016)	Dioxins	Vietnam	2008–2009	217	Breast milk	1 month after birth	First 3 years of life
Tan et al. (2009)	POPs (PCB, PBDE, and OCPs)	Singapore	2006	41	Cord blood	At birth	Infant
Valvi et al. (2012)	OCs (PCBs, DDE, and DDT)	Spain	1997–1998	344	Cord blood	At birth	6.5 years
Vreugdenhil et al. (2004)	PCBs	Netherlands	1990–1992	104	Plasma	Last months of pregnancy	At 9 years of age
Wolff et al. (2007)	DDE, PCB	USA	1998–2002	404	Plasma	Third trimester	Infant
Gladen et al. (2000)	PCBs and DDE	USA	1992	594 (316 girls and 278 boys) children	Breast milk, maternal blood, cord blood, and placenta	Not indicated	At 12 years
Ribas-Fitó et al. (2002)	HCB	Spain	1997–1999	98	Maternal serum and cord serum	At delivery	Infant
Swan et al. (2005)	Phthalate	USA	1999–2002	85	Urine	Mean GW28.3	At 2–36 months

[a]Cases, children born to mothers who survived HCB episode; controls, children born to mothers who continuously lived in the same area
[b]Cases, mothers-neonate dyads with neural tube defects; control, mothers-neonate dyads without congenital anomalies. *GW* gestation week, *EDD* expected day of delivery
[c]Blood for PCBs, PBDE, and OCPs, urine for BPA, and phthalates and breast milk for BPA and phthalates
[d]Cases, mother-children with sensorineural hearing loss; controls, children selected at random
[e]At registration, every 8 weeks during pregnancy, at delivery, and 6 weeks postpartum
[f]Cases, boys with cryptorchidism, hypospadias, and polythelia (extra nipple); controls, healthy babies
[g]Cases, sons with cryptorchidism and hypospadias; control, sons with neither condition
[h]Cases, mothers who delivered neural tube defect infants; controls, mothers who delivered healthy infants
[i]Cases, mothers of cryptorchid boys; controls, mothers with healthy boys

Table 4 Summary of number of studies per chemical and their distribution across effects on the offspring

Chemicals	Total	Reproductive effects	Immunological effects	Metabolic effects	Neurological effects	Growth and development effects
Animal studies ($N = 113$)		$n = 52$	$n = 4$	$n = 20$	$n = 31$	$n = 6$
Phenols (BPA, NP, and OP)	43	16	2	10	12	3
Phthalates	24	16	0	4	4	0
Dioxins	18	9	1	1	7	0
PCBs	12	4	1	1	5	1
PBDEs	2	1	0	0	1	0
Other persistent chemicals (FV, VN, TBT, TPT, DDT)	9	6	0	4	1	1
Multiple (2+)	2	0	0	0	1	1
Human studies ($N = 73$)		$n = 16$	$n = 7$	$n = 11$	$n = 9$	$n = 30$
Phenols (BPA and NP)	7	1	0	1	1	4
Phthalates	7	2	0	0	0	5
Persistent chemicals (PCBs, PBDEs, DDT, DDE, HCB, OCPs)	43	11	3	7	5	17
PFASs	6	0	2	2	0	2
Dioxins and dioxin-like compounds	4	1	1	1	1	0
Multiple (2+)	6	1	1	0	2	2

maternal chemical levels at different pregnancy durations. For the persistent chemicals' quantification, maternal blood was mostly used ($n = 34$) except for few studies which used breast milk ($n = 7$), breast milk and blood ($n = 1$), cord blood ($n = 4$), cord blood and breast milk ($n = 2$), maternal blood and cord blood ($n = 3$), and other ($n = 8$). Most of these studies ($n = 34$) were done in European countries, followed by North America ($n = 25$), Asia ($n = 13$), and Australia ($n = 1$). None of the studies was done in Africa. Details of the adverse health outcomes on the offspring exposed to EDCs in utero or during lactation are described below. The adverse health effects are summarized in Table 5 (animal studies) and Table 6 (human studies).

3.2 Animal Experimental Studies

3.2.1 Exposure to Nonpersistent Chemicals in Rodents

Phenols (BPA, NP, and OP)

Phenols are environmental chemical contaminants which belong to category 1 of EDCs (clear evidence of endocrine-disrupting activity) (Errico et al. 2017). They interfere with endocrine system functions by binding to estrogen receptors (ERs) and act competitively toward natural hormones. Several studies have reported potential adverse health effects on the exposed offspring.

Reproductive Effects

Rodent experimental studies have been performed on the effects of perinatal exposure to BPA on mammary gland showing the development of proliferative lesions in the glands of female rat offspring. Most of the reported effects occur at the lowest doses investigated. At PND90, mammary gland adenocarcinomas were reported in female rat offspring exposed to 250 μg/kg-BW/day which is an environmentally relevant level (Acevedo et al. 2013). Intraductal hyperplasia was also reported in female adult offspring (PND400) exposed perinatally to a tenfold lower dose (25 μg/kg-BW/day) (Mandrup et al. 2016). These studies suggest that low-dose exposure to BPA can affect mammary gland development in female rat offspring. For the NP-exposed female pups, advanced lobular development of their mammary glands was observed on PND22 in a group exposed to high dose (100 mg/kg-BW/day), and not low dose (Moon et al. 2007). Noteworthy, these three studies have a big variation in the exposure period. In the first study, dams were exposed once at GD18, while the duration of exposure in the second study was long, starting at GD7 to PND22 (birthday excluded). The NP-exposed dams were exposed during the late stages of pregnancy only (GD15–19). These variations might have significantly affected outcomes.

Table 5 Summary of adverse health effects from animal experimental studies

EDCs	Adverse health effects
Reproductive effects	
BPA	• ♀ induced malignant mammary gland tumors (Acevedo et al. 2013) • ♀ induces a delay in the functional differentiation of the mammary gland in F1 and ↑ body weight gain of F2 (Altamirano et al. 2015) • Early vaginal opening (Honma et al. 2002) • ♂ impaired sexual performance (Jones et al. 2011) • ♀↑ mammary development (Mandrup et al. 2016) • ♀↑ in postimplantation loss and ♂↓ sperm count and motility in the F1–F3 (Salian et al. 2009) • Vaginal morphological changes at postpubertal (Schönfelder et al. 2002)
OP	• ♀ disrupted vaginal cyclicity, ♂↓ seminiferous tubule diameter and testis (Pocock et al. 2002)
NP	• ↑ mammary development (♀) (Moon et al. 2007)
DEHP	• ♂↓ fertility prematurely (Barakat et al. 2017) • ♂↑ nipple retention, ↓ sperm motility, and ↑ sperm count (Moore et al. 2001) • ♂ disruption of testicular function in F1–F4 (Doyle et al. 2013) • ♂↓ AGD (Mylchreest et al. 2000) • ♀↓ oocyte quality and embryonic developmental competence in F1–F3 (Pocar et al. 2017) • ♂↓ in daily sperm production and ↑ serum testosterone concentration (Andrade et al. 2006)
DINP	• ♂↑ nipple retention, ↓ sperm motility, and ↑ sperm count (Boberg et al. 2011)
DBP	• Deterioration in sperm quality parameters at 50 mg/kg (Ahmad et al. 2014) • ♂↓ AGD, ♂↑ incidence of hypospadias and cryptorchidism (Kim et al. 2010) • ♂↓ penile length and ↑ incidence of severe hypospadias (Li et al. 2013a) • ♂↓ AGD (Ma et al. 2017)
BBP	• Deterioration in sperm quality parameters at 100 mg/kg (Ahmad et al. 2014) • ♂↓ AGD, undescended testes (Ema and Miyawaki 2002) • ♂↓ AGD (DeBartolo et al. 2016)
MBuP	• ♂↓ AGD, undescended testes (Ema and Miyawaki 2001)
TCDD	• ♀ impaired mammary gland maturation (Fenton et al. 2002) • ♂↓ AGD and testis weights (Jin et al. 2008) • Early vaginal opening and first estrus (Kakeyama et al. 2008) • ↑ incidence of mammary tumor in HFD-fed mice (La Merrill et al. 2010) • ♂↓ urogenital complex, ventral prostate weights, and AGD (Ohsako et al. 2002) • ♂↓ sperm quality and fertility in F1–F3 (Sanabria et al. 2016) • ♀↓ fertility and ↑incidence of PTB in F1–F4 (Bruner-Tran and Osteen 2011)
Vinclozolin	• ♂↓ spermatogenetic capacity of the adult in F1–F4 (Anway et al. 2006) • ♂ testicular maldescent and ↓ AGI (Shono et al. 2004)
PCBs	• ↓ sperm-fertilizing ability in vitro at 16 and 45 weeks of age (Fielden et al. 2001) • ↓ sperm and spermatid numbers and impairment of daily sperm production (Kuriyama and Chahoud 2004) • Altered uterine and ovarian weight in F2 adult (Steinberg et al. 2008) • ♀ earlier puberty onset, ♂ delayed testicular descent (Colciago et al. 2009)
PBDEs	• ♂ ↓AGD, delay in preputial separation, and ↓mean testosterone concentration (Kodavanti et al. 2010) • ♂↓ circulating sex steroids in male offspring at weaning and in adulthood, ↓AGD (Lilienthal et al. 2006) • ♀ delayed puberty onset (Lilienthal et al. 2006)

(continued)

Table 5 (continued)

EDCs	Adverse health effects
TBT	• ♀ early puberty and impaired estrous cyclicity (Si et al. 2012a) • ↓sperm counts and motility (Si et al. 2013)
Neuro- and behavioral effects	
BPA	• Enlarged anteroventral periventricular nucleus (Arambula et al. 2017) • ♀↑ sweet preference in F1 and F2 (Boudalia et al. 2014) • Disruption of global metabolism (Cabaton et al. 2013) • ↑ adiposity and systolic blood pressure and impaired glucose homeostasis (Cagampang et al. 2012) • ♀ masculinization of female behavior, ♂ an intensification of male behavior (Dessì-Fulgheri et al. 2002) • ♀ masculinization of spatial learning and ↓ sperm count (Hass et al. 2016) • Neurocognitive deficit in F2 (Jang et al. 2012) • ♂ disrupted normal spatial learning and exploratory behaviors and induced anxiety-like behaviors (Jašarević et al. 2013)
OP	• ♂ ↑ sexual arousal, ♀↑ sexual motivation and motor activity (Pocock et al. 2002)
DEHP	• ↑ anxious behavior and impaired recognition memory (Barakat et al. 2018) • Impaired locomotion activity and spatial memory (Dai et al. 2015) • ↑ anxiety- and depressive-like behaviors in puberty and adulthood (Xu et al. 2015) • Induced neurotoxicity in immature offspring (Li et al. 2013b)
TCDD	• ♂ hyperactivity and ↓ duration of proximity and social contact (Anh et al. 2013) • Behavioral inflexibility, compulsive repetitive behavior, and ↓ competitive dominance (Endo et al. 2012) • Induced hyperactive behavior (Hojo et al. 2008) • ♂ demasculinization (Ikeda et al. 2005) • Perturbed paired-associate learning (Kakeyama et al. 2014) • ♂ induced hyperactivity and socioemotional deficits (Nguyen et al. 2013) • ♂ delayed fetal brain growth and neurodevelopment in early stage (Nishijo et al. 2007)
PCBs	• ♂ anxiety-like behaviors (Orito et al. 2007; Gillette et al. 2017) • ♂ ↑ sweet preference in adults (Kaya et al. 2002) • ↓ walking speed in the open-field test and a prolonged time to reach the platform in the water maze test (Sugawara et al. 2006)
PBDEs	• ♂↑ sweet preference in adults (Lilienthal et al. 2006)
TBT	• Delay in cliff-drop aversion (TBT) (Si et al. 2012b)
Growth and development effects	
BPA	• ♀ elongated femur, ♂↑ cortical thickness (Lejonklou et al. 2016) • ♂↓ femurs length (Lind et al. 2017) • Growth retardation (Takagi et al. 2004.)
NP	• Growth retardation (Takagi et al. 2004)
TBT	• Suppressed the growth and delayed eye opening (Makita et al. 2004)
TPT	• Delayed eye opening and physical maturation (Grote et al. 2007)
Metabolic effects	
BPA	• ♂↓ glucose tolerance, ↑ insulin resistance (Alonso-Magdalena et al. 2010; Angle et al. 2013) • ♀ accelerated the insulitis and spontaneous diabetes type 1 development (Bodin et al. 2014) • Induce changes in fatty acid metabolism (Dunder et al. 2018)

(continued)

Table 5 (continued)

EDCs	Adverse health effects
	• ♂ impaired glucose tolerance at 6 weeks and 6 months (Galyon et al. 2017) • Glucose intolerance and insulin resistance in F2 (Li et al. 2014) • Hyperglycemia with insulin resistance, ↑ oxidative stress, and ↓ adiponectin production (Song et al. 2014) • Impaired body weight and body composition (Rubin et al. 2016)
NP	• ♂ induced glucose metabolism disorder (Yang et al. 2017)
DEHP	• ↑ serum leptin, insulin, lipid, and fasting glucose concentrations (Gu et al. 2016) • ↑ blood pressure (Lee et al. 2016) • ♀↑ blood glucose, ↓ serum insulin and impaired glucose tolerance, and insulin secretion (Lin et al. 2011) • ↓ systolic and diastolic systemic arterial pressures (Martinez-Arguelles et al. 2013)
TCDD	• ↑ susceptibility to renal fibrosis and hypertension in adulthood (Aragon et al. 2008)
Vinclozolin	• Anemia late in pregnancy and glomerular abnormalities in F1–F3 (Nilsson et al. 2008)
PCBs	• Altered hormones and body weight in F2 and F3 (Mennigen et al. 2018)
DDT	• ♀↓ core body temperature, impaired cold tolerance, ↓energy expenditure, ↑ body fat (La et al. 2014)
TBT	• Increased fat mass when switched to a higher fat diet and impaired fat mobilization during fasting (Chamorro-Garcia et al. 2012) • Increased white adipose tissue fat depots and induced a phenotype resembling nonalcoholic fatty liver disease (Chamorro-García et al. 2013)
Immunologic effects	
BPA	• ♂ induced islet inflammation in F1 and F2 (Bansal et al. 2017) • ♀ intestinal barrier dysfunctions in adulthood (Braniste et al. 2010)
TCDD	• ↑ incidence of airway obstruction (Hamm et al. 2000)

AGD anogenital distance, *AGI* anogenital index, *HFD* high-fat diet, *PTB* preterm birth, *DDT* dichlorodiphenyltrichloroethane, *TBT* tributyltin, *TPT* triphenyltin, *MBuP* monobutyl phthalate, *DBP* di-*n*-butyl phthalate, *BBP* benzyl butyl phthalate, *PCBs* polychlorobiphenyls, *TCDD* 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, *PBDEs* polybrominated diphenyl ethers

The reproductive effects following perinatal exposure to BPA are sex-dependent and are dominant in female offspring. Interestingly, while studies have reported adverse reproductive effects in the exposed female offspring, similar studies did not record any effect in the male. Authors have reported early vaginal opening in BPA-exposed offspring (Honma et al. 2002). But early vaginal opening did not have a significant impact on general female pubertal development and reproductive functions (Kwon et al. 2000). However, there are reports of vaginal morphological changes at a postpubertal age in BPA-exposed and disrupted vaginal cyclicity in OP-exposed offspring (Pocock et al. 2002; Schönfelder et al. 2002). Several studies did not record significant reproductive effects in the first-generation offspring exposed to phenolic chemicals (Kobayashi et al. 2002; Ichihara et al. 2003; Kimura et al. 2006; Ryan et al. 2010; LaRocca et al. 2011; Spörndly-Nees et al. 2018). However, a lack of adverse effects in the first generation does not guarantee the same results in subsequent generations.

Table 6 Summary of adverse health effects from human epidemiological studies

EDCs	Adverse health effects
Reproductive effects	
BPA	• ♂ ↓ AGD at 12 months (Sun et al. 2018)
DEHP metabolites	• ♂ ↓ AGI and testicular descent impaired (Swan et al. 2005)
Organochlorides	• ↑ risk of congenital cryptorchidism (Damgaard et al. 2006; Brucker-Davis et al. 2008)
PCBs	• ♀↓ fecundability (Han et al. 2016)
PBDEs	• ♂ ↓ AGI (García-Villarino et al. 2018) • ↑ risk of congenital cryptorchidism (Main et al. 2007)
Neuro- and behavioral effects	
BPA	• ♀Externalizing behaviors at 2 years (Braun et al. 2009) • Social impairment (Lim et al. 2017)
DDE	• ↑ risk of autism (Brown et al. 2018) • ↑ risk of neural tube defects (Kalra et al. 2016)
PCBs	• ↓ reaction times and scores on the Tower of London (Vreugdenhil et al. 2004) • Lower psychomotor scores at 2 years (Gascon et al. 2013)
Dioxins	• ♂ ↓ expressive communication, composite, and gross motor scores (Tai et al. 2016)
Growth and development	
BPA	• ↓birth weight and head circumference (Mustieles et al. 2018) • ↑ waist circumference and BMI (Valvi et al. 2013; Vafeiadi et al. 2016)
NP	• ↑risk of low neonatal weight (Chang et al. 2013)
MCNP	• ↑ femoral length and child height (Botton et al. 2016)
DEHP and MEP	• ♂ ↓language scores (Olesen et al. 2018)
BBP and DEHP	• ↑ incidence of croup in infants up to 12 months (Shu et al. 2018)
DEHP metabolites	• ↑birth weight (Zhu et al. 2018)
PBDEs	• ↓ cognitive abilities up to 8 years (Braun et al. 2017)
DDE	• ♂↑ height at puberty (Gladen et al. 2000)
PFASs	• ↓ vocabulary score at 15 months and language score at 38 months (Jeddy et al. 2017) • ↓ bone size and mass (Jeddy et al. 2018)
PCBs	• ♀↑ risk of language delay at age 3 years (Caspersen et al. 2016)
Dioxins	• ♀↑ early infant growth and BMI in school-age girls (Iszatt et al. 2016)
PBB	• ↑ odds of a below-median Apgar score at 1 and 5 min (Terrell et al. 2015) • ♀ earlier age at menarche and earlier pubic hair stage (Blanck et al. 2000)
Immunologic effects	
PCB and HCB	• ↑ risk of asthma at 20 years (Hansen et al. 2014)
PFASs	• ♀↑ risk of childhood atopic dermatitis in first 24 months of life (Chen et al. 2018)
Dioxins	• ↑ frequency of wheezing up to 7 years (Miyashita et al. 2018) • ↑ risk of otitis media at 18 months (Miyashita et al. 2011)
Metabolic effects	

(continued)

Table 6 (continued)

EDCs	Adverse health effects
Organochlorides	• Induces clinical porphyria cutanea tarda (Jarrell et al. 2002) • ♀↑ risk of obesity (Smink et al. 2008; Valvi et al. 2012; Vafeiadi et al. 2015) • Rapid weight gain in the first 6 months (Mendez et al. 2011)
PFASs	• ↑ risk of obesity (Lauritzen et al. 2018) • ↓ adiposity (Starling et al. 2017)

AGD anogenital distance, *AGI* anogenital index, *MEP* monoethyl phthalate, *MBP* mono-*n*-butyl phthalate, *MBzP* monobenzyl phthalate, *MiBP* monoisobutyl phthalate, *POPs* persistent organic pollutants, *MCNP* monocarboxyisononyl phthalate, *BBP* benzyl butyl phthalate, *PBB* polybrominated biphenyls, *HCB* hexachlorobenzene, *PFASs* perfluoroalkyl substances, *PBDEs* polybrominated diphenyl ethers, *PCBs* polychlorobiphenyls, *BPA* bisphenol A

Few multigeneration studies have been identified for phenols. First-generation offspring exposed to low BPA dose (0.6 μg/kg-BW/day) have been reported with a delay in the functional differentiation of the mammary gland during secretory activation. The consequence of this effect was the impairment of F1 milk lipid content and fatty acid composition which resulted in an increased body weight gain of F2 suckling rats (Altamirano et al. 2015). Meanwhile, developmental exposure of F0 to NP (below NOEL) in drinking water affected neonatal birth weight in the F1 pups. However, the F3 pups recovered to normal (F0) state (Chang et al. 2014). This signifies that if preventive measures are employed in the subsequent generation, no inherited effects will be observed. This is contrary to offspring exposed to 1.2 and 2.4 μg/kg BW/day of BPA. In this study, the offspring presented with a significant increase in postimplantation loss and a decrease in litter size, sperm count, and sperm motility in the F1. These effects were amplified in the F2 and F3 generations (Salian et al. 2009).

Effects on the Metabolic System

The effects of perinatal exposure to BPA in rodents are sex-specific and in most cases are influenced by the precise window of BPA exposure. Male offspring exposed to BPA during the period of differentiation of preadipocytes (GD6–16) have been reported with impaired glucose tolerance, insulin resistance, and altered blood parameters at doses even below "no observed adverse effect level" (NOAEL) (Alonso-Magdalena et al. 2010; Cagampang et al. 2012; Angle et al. 2013; Galyon et al. 2017). At PND100, male offspring exposed in utero to BPA in drinking water developed hyperglycemia with insulin resistance, increased oxidative stress, and decreased adiponectin production (Song et al. 2014). In addition to BPA, NP perinatal exposure was also reported to induce glucose metabolism disorder in male rat offspring (Yang et al. 2017).

Few studies have documented an impairment in the metabolic functions of female offspring exposed to phenols. During adulthood, the offspring presented insulitis and spontaneous diabetes type 1 after in utero and breast milk BPA exposure (Bodin

et al. 2014). Similarly, the increase in body weight was observed at a very low BPA dose (0.5 μg/kg-BW/day) in adult female offspring (Dunder et al. 2018) and at environmental relevant dose in both sexes (Rubin et al. 2016). There is some evidence of the multigenerational transfer of metabolic effects resulting from exposure to phenolic chemicals. Second-generation offspring (F2) of pups exposed to BPA from conception day to weaning also had glucose intolerance and insulin resistance (Li et al. 2014). Surprisingly, in a later study, compromised maternal metabolic milieu was reported in both F1 and F2. But the effects observed in F1 cannot account for all of the observed multigenerational phenotypes (Susiarjo et al. 2015).

Generally, animal studies have made it clear that perinatal exposure to phenolic chemicals, particularly BPA, impairs metabolic processes that are precursors of overweight, obesity, altered glucose/insulin homeostasis, diabetes, and cardiovascular diseases in adulthood. The effects are more expressed in male offspring.

Neurobehavioral Effects

The endocrine and nervous systems are closely connected; therefore abnormal alterations in the functions of one system automatically link to the other. The brain is highly vulnerable to chemical exposures, and changes in its functions can cause widespread disruption in hormone receptors and nerve signals, thus affecting infant brain development (Xu et al. 2010). The effects from phenolic chemicals exposure can occur even at doses far below the NOAEL. For example, at as low as 0.025 μg BPA/kg-BW/day, impairment of brain function was reported in the PND2 and 21 exposed offspring (Cabaton et al. 2013). In addition, in utero exposure to 2.5 mg BPA/kg-BW/day was associated with an enlarged anteroventral periventricular nucleus (AVPV) of the offspring's brain (Arambula et al. 2017). Surprisingly, dietary exposures of up to 150 mg BPA/kg-BW/day throughout pregnancy and lactation did not result in neurotoxic action in rat offspring (Stump et al. 2010). This might be resulting from errors in calculating target exposure doses from dietary exposure.

Phenols also exert sex-specific behavior alterations in exposed offspring. At low dose (40 μg/kg/day), BPA-exposed offspring presented masculinization of female behavior, but at a high dose (400 μg/kg/day), no effects were observed in the females. Rather, there was an intensification of male behavior in males (Dessì-Fulgheri et al. 2002). A similar effect was observed in offspring exposed to a dose range of 25–50 mg/kg-BW/day who presented with masculinization of spatial learning in females (Hass et al. 2016). In a study using outbred deer mice, F1 exposed to 50 μg, 5 mg, and 50 mg/kg-BW/day had disrupted spatial learning and exploratory behaviors and produced anxiety-like effects in male but not in female mice (Jašarević et al. 2013). The effects were most dominant in the mid and upper dose. Temporary activation of aggressive behavior in mice at 8 weeks of age were also observed in BPA-exposed offspring (Kawai et al. 2003). Surprisingly, SD rat offspring exposed maternally to 50 and 500 μg/kg/day from 10th day of gestation to 14th day of lactation observed no significant behavioral alterations in all the tests

(Kuwahara et al. 2013). The pattern of effects observed in these studies indicates that the effect of BPA on behavior patterns of offspring is not only sex-specific but is also non-monotonic in terms of dose response.

In terms of neurodevelopmental and behavioral effects across generations, we managed to acquire two studies only. The first study observed adverse effects on both F1 and F2 generations (Boudalia et al. 2014). Similarly, the neurocognitive deficit in terms of memory retention was observed in F2 of C57BL/6 mice (Jang et al. 2012). Although there is an inadequate amount of evidence, we cannot rule out the multigenerational transfer of effects as both studies reported significant effects.

Other Effects

Very few studies have explored the role of maternal BPA on the impairment of the immune system and the offspring's growth and development. Still, the outcomes suggest positive associations. Offspring exposed to BPA, as low as 5 mg/kg/day, suffered intestinal barrier dysfunctions in adulthood. The effects were presented by a decrease in colonic paracellular permeability in the female offspring (Braniste et al. 2010). There is also evidence of immunotoxic effects in the F2 generation without additional exposure to F1. BPA-induced islets inflammation observed in the F1 exposed offspring persisted to the F2 generation (Bansal et al. 2017).

Little evidence is available linking perinatal BPA exposure and offspring's physical growth impairment. Both low dose (25 μg BPA/kg-BW/day) and high dose (5,000 μg BPA/kg-BW/day) elicited femur elongation, particularly in females. In male offspring, the increased cortical thickness was only observed in the small dose group (Lejonklou et al. 2016). This adds up to effects that skew toward low-dose exposures.

Phthalates

Phthalates are a class of nonpersistent chemical compounds that are used in a multitude of consumer products; and exposure to them occurs through ingestion, dermal, or inhalation. Although they have short biological half-lives (<24 h), repeated, episodic, and long-term exposures to them occur (Hipwell et al. 2019). Phthalates can cross the placenta to induce developmental defects in animals and may exert similar effects in humans (Gray et al. 2000). The effects of perinatal exposure to phthalates in rodents are summarized below.

Reproductive Effects

Findings from a meta-analysis of 19 experimental animal studies have shown that exposure to antiandrogenic DEHP is associated with reduced anogenital distance (AGD) with a dose-response gradient (Dorman et al. 2018). Phthalates are also responsible for decreasing male fertility (Barakat et al. 2017) by deteriorating sperm quality (Ahmad et al. 2014) and sperm count (Boberg et al. 2011). Effects related to

disruption of testicular function have been reported to persist up to the F4 offspring (Doyle et al. 2013). Reduction of sperm count can be up to a rate of 19–25% reduction in daily sperm production which is above the recommended cutoff point of 20% using rat animal models (Andrade et al. 2006). Some female fertility-related effects, which are not limited to the F1 offspring, have been reported. DEHP-exposed female offspring were recorded with reduced oocyte quality and embryonic developmental competence in F1 and were transferred up to the F3 offspring (Pocar et al. 2017).

Studies have also demonstrated an increase in the incidence of other reproductive tract malformations particularly inguinal cryptorchidism, undescended testes, and hypospadias (Ema and Miyawaki 2001, 2002; Moore et al. 2001; Kim et al. 2010; Li et al. 2013a). Both BBP and MBuP have been identified to be associated with undescended testes in male offspring in a dose-dependent manner (Ema and Miyawaki 2001, 2002; Moore et al. 2001). The incidences of hypospadias and cryptorchidism have been reported to increase in the higher doses as it was recorded in DBP (700 mg/kg-BW/day)-treated group (Kim et al. 2010) as well as DEHP (1,000 mg/kg-BW/day)-treated group (Li et al. 2013a). At low doses of DBP (100 mg/kg-BW/day) and DEHP (3 and 30 mg/kg-BW/day), no significant reproductive changes were observed in female and male rat offspring, respectively (Guerra et al. 2010; Carbone et al. 2012).

Metabolic Effects

Phthalates are considered as potential obesogens because of their ability to promote the development of obesity through interference with critical pathways associated with energy balance, adipogenesis, and lipid metabolism. They are associated with impaired glucose tolerance and insulin secretion in female offspring (Lin et al. 2011). The stated effects are expressed by elevated serum leptin, insulin, lipid, and fasting glucose concentrations (Gu et al. 2016). Some cardiometabolic effects have also been observed in the exposed offspring, but the results are contradictory. Eight-week-old offspring exposed to 30 mg/kg-BW/day DEHP had an increased blood pressure (Lee et al. 2016). Meanwhile, high-dose exposure (300 mg/kg-BW/day) resulted in a reduced systolic and diastolic systemic arterial pressures in 28-week-old offspring (Martinez-Arguelles et al. 2013). The differences might be attributed to methodological differences between the two studies. Differences in rodent species (C57BL/6 mice vs SD rats), exposure duration (4 weeks before mating to weaning versus GD14–birth), dose, and outcome measure period (young vs old) might have caused the variations.

Neurobehavioral Effects

The antiandrogenic nature of DEHP is also linked to impairment of neuronal development in exposed offspring resulting in abnormal neurobehavior. Under similar experimental models, exposure of ICR mice offspring to DEHP resulted in an impairment in their pubertal and adulthood behaviors (Xu et al. 2015; Dai et al.

2015). At higher DEHP exposure doses, CD-1 mice offspring had elevated anxious behavior and impaired recognition memory in adulthood (Barakat et al. 2018). DBP is also involved in the induction of neurotoxic effects in immature offspring, but it has no influence on mature offspring (Li et al. 2013b). The mechanism behind the ability of DEHP to elicit neurobehavioral effects in perinatally exposed offspring is still under debate.

3.2.2 Exposure to Persistent Chemicals in Rodents

There are about 30 chemicals listed in the Stockholm Convention declared for banning in 2001 because of their resistance to degradation and persistence in the environment (UNEP 2010). These chemicals, despite the international intent for banning them, still exist in the food chain of animals and humans. Both humans and animals are in a constant exposure to cocktails of persistent chemicals. Rodent studies have been conducted to simulate maternal human exposures to persistent and nonpersistent chemicals and to assess their consequential outcomes in the subsequent generations of offspring. Unfortunately, most of these studies have focused on nonpersistent chemicals, while very few have focused on persistent chemicals.

Persistent Organochloride Compounds

PCBs are the most explored among organochloride compounds. Maybe it was because of their extensive use in various industrial and commercial applications before they were banned. Studies have provided evidence of impairment in the offspring's reproductive physiology, fertility, and fecundity. Male offspring fertility has been reported to be compromised after in utero exposure to PCBs, and the effects are manifested by impairment of daily sperm production (Kuriyama and Chahoud 2004). Loss of sperm's fertilizing ability (in vitro) at 16- and 45-week-old C57BL6 mice offspring was also reported after PCB exposure (Fielden et al. 2001). No effects were observed on the AGD among the exposed male offspring (Fielden et al. 2001; Kobayashi et al. 2009). Similar effects were observed in female offspring exposed to PCB (A1221), and more effects were recorded in the F2 generation compared to the F1 generation (Steinberg et al. 2008). This might be an indication of bioaccumulation of chemical levels across generations.

Perinatal exposure to organochloride compounds has resulted in neurobehavioral effects in the exposed rodent's offspring. To mimic the cocktail nature of persistent chemicals in the environment, SD rat offspring were exposed to a mixture of 14 PCBs and 11 OCPs in the whole period of gestation and lactation, and neurobehavioral endpoints were measured. Several neurobehavioral effects were observed in a dose-dependent manner (Bowers et al. 2004). Exposure to PCBs is also associated with anxiety-like behaviors in male offspring (Orito et al. 2007; Gillette et al. 2017) and feminization of sweet preference (Kaya et al. 2002).

Other adverse health effects associated with early-life exposure to organochloride compounds include metabolic, immune, and growth disturbances. Female offspring exposed to DDT exhibited a reduced core body temperature, an impaired cold tolerance, a decreased energy expenditure, and a transient increase in early-life body fat (La Merrill et al. 2014). On the other hand, offspring exposed to PCB (A1221) does not only disrupt growth and serum hormones in the F1, but the effects cut across to the F2 and F3 generations (Mennigen et al. 2018). Effects on growth alter age at puberty onset as well. Exposed rat offspring expressed early puberty onset in female and delayed testicular descent in male (Colciago et al. 2009).

Dioxins

In the offspring's reproductive development, TCDD exposure is associated with reduced AGD in the male during development (GD15 and 18) (Ohsako et al. 2002) and later in life (PND30 and 60) (Jin et al. 2008). In the female offspring, exposures are associated with impairment in the maturation of mammary glands (Fenton et al. 2002), acceleration of mammary tumor incidence in offspring fed with a high-fat diet (La Merrill et al. 2010), and earlier vaginal opening and first estrus appearance. These were observed 4–7 days earlier than normal (Kakeyama et al. 2008). At low (0.1–1 μg/kg-BW/day) and high (10 μg/kg-BW/day) TCDD doses administered by oral gavage on GD15, both male and female offspring had impaired fertility. These effects were transferred to the next generations, up to the F3 (male) and F4 (female) generations (Bruner-Tran and Osteen 2011; Sanabria et al. 2016).

With the exception of one study (Hojo et al. 2008), all studies on neurobehavioral effects of TCDD exposure have been done in male offspring. Exposed pups were subjected to various behavior and learning tests. Hyperactivity behavior (Hojo et al. 2008; Anh et al. 2013; Nguyen et al. 2013), compulsive repetitive behavior (Endo et al. 2012), and paired social learning (Kakeyama et al. 2014) were perturbed after in utero exposure. The effects might be associated with the reported delayed fetal growth and neurodevelopment in early stages of development (PND4–14) (Nishijo et al. 2007).

Other Persistent Chemicals

There are other persistent chemicals that render adverse health effects after developmental exposure. All of them present similar kind of effects in the exposed offspring; and these effects cut across reproductive, growth, metabolic, and neurobehavioral development. Flame retardants (PBDEs), antiandrogenic dicarboximide fungicide (vinclozolin, VZ), estrogenic synthetic pyrethroid insecticide (fenvalerate, FV), and organotin compounds (TBTCl and TPTCl) are all linked to adverse reproductive and other health outcomes in the exposed offspring. PBDEs, TBTCl, and VZ are linked with impairment of male reproductive physiology. PBDEs and VZ have been reported to reduce AGD and impair spermatogenesis in male offspring

(Shono et al. 2004; Lilienthal et al. 2006; Kodavanti et al. 2010). PBDEs are also associated with a delay in preputial separation, an effect that was not observed in the FV-exposed offspring (Nassr et al. 2010; Kodavanti et al. 2010).

SD rat male offspring exposed to 100 mg/kg-BW/day VZ from embryonic (E) days 8 to 14 presented with reduced spermatogenetic capacity in four generations (F1–F4) indicating impaired fertility (Anway et al. 2006). Meanwhile, when the same dose was given to Wistar rats from GD6 to 15, no effects were observed in male offspring sexual development in all the F1–F4 generations (Schneider et al. 2008). This might be due to the differences in the exposure period as a window of sensitivity for antiandrogenic effects is normally from days 16 to 20 post coitus (Schneider et al. 2008).

Four studies from two authors have explored the effects associated with exposure to organotin compound (TBTCl). Wistar rats exposed to 25 ppm TBT (approximately 2 mg/kg) in the diet in the whole period of gestation and lactation resulted in growth suppression and delayed eye opening in their offspring (Makita et al. 2004). Meanwhile, oral gavage of Chinese Kun Ming mice with low doses of 1, 10, and 100 μg TBTCl/kg-BW/day from GD6 to PND22 resulted in various dose-specific effects. 10 and 100 μg/kg exposed offspring expressed a delay in cliff-drop aversion response in the behavior test and altered patterns of estrous cyclicity in adulthood (Si et al. 2012b). Meanwhile, at 100 μg/kg TBTCl retarded the testes descent of male offspring (Si et al. 2012b). Similarly, exposed offspring presented early puberty and impaired estrous cyclicity in female (Si et al. 2012a) and decreased sperm counts and motility in male (Si et al. 2013). These findings suggest that reproductive and neurobehavioral toxicity might be good markers of low-dose TBTCl exposure.

Chamorro-Garcia and colleagues had a series of animal experiments to test whether prenatal TBT exposure elicit obesogenic effects that can be transferred to subsequent generations without additional exposure. After a high-fat diet challenge, the F4 offspring presented an increased fat mass in adulthood. Additionally, during the fasting period, fat mobilization was significantly impaired (Chamorro-Garcia et al. 2012). In another experiment, TBT induced a phenotype resembling nonalcoholic fatty liver disease in the F3 of the in utero-exposed F1. They also observed an increase in the white adipose tissue fat depots (Chamorro-García et al. 2013). Altogether, these results suggest that early-life exposure to obesogens might have long-lasting effects even without additional environmental exposures.

3.3 Human Epidemiological Studies

3.3.1 Exposure to Nonpersistent Chemicals

Studies have been identified focusing on in utero and/or lactational exposure to phenols (BPA and NP) and phthalates. All studies used maternal urine samples because both phenols and phthalates have short half-lives. Urine samples were taken at different pregnancy durations ranging from the first trimester to the day of

delivery. To get a better association between maternal chemical levels in urine and adverse health effects on the exposed offspring, most of the articles in this review used multiple samples. However, some studies used only one sample to establish the association between phthalates and BPA and birth weight indices and children growth, respectively (Swan et al. 2005; Vafeiadi et al. 2016). With such studies, the strength of the association is questionable.

Since AGD is associated with multiple semen parameters, it is considered to be the most sensitive marker in predicting the effect of antiandrogenic EDCs in human populations (Mendiola et al. 2011). AGD has been reported to be affected by exposure to EDCs during the masculinization programming window (Scott et al. 2008). Prenatal androgen exposure or hyper-androgenic environment have the potential to modify female offspring's reproductive tract during in utero development, resulting in AGD impairment (Mira-Escolano et al. 2014). A recent systematic review and meta-analysis study reported an association between decreased AGD and maternal urine DEHP metabolites concentrations (Dorman et al. 2018). To support these findings, low levels did not have any significant effect on AGD but had impaired testicular descent (Jensen et al. 2015). A similar association was observed in 12 months infant boys born to mothers with higher urine concentration of BPA (Sun et al. 2018).

Although the amount of evidence is limited, researchers established the impact of perinatal BPA on impairment of children's neurobehavioral development. Until 2009, there were no studies that had examined the association between BPA and neurobehavioral development in children. The first study to establish association was done on 249 USA mothers and their children which reported externalizing behaviors in 2-year-old girls (Braun et al. 2009). Later, in 2015, social impairments in 4 years children were reported about Korean children who were produced by mothers with high BPA urine concentration (Lim et al. 2017). Recently, delayed neurodevelopment with increasing urinary phthalate metabolites and BPA has been reported in 13–24 months toddlers in the Korean (CHECK) cohort (Kim et al. 2018). However, the available evidence is not enough to confirm the direct cause-effect relationship. Further studies are required to evaluate the effects of perinatal exposure to nonpersistent pollutants on children's behavior and their associated underlying mechanisms.

Sixty-four percent (9/14) of the studies on nonpersistent chemicals have focused on the impacts of high levels of phthalates and phenols on children's growth and development from gestation to infancy. Increased fetal height, during the third trimester, has been observed to be positively associated with exposure to DEHP (Botton et al. 2016). Contrary to these findings, a USA-based cohort study found no association between phthalate metabolites and infant length (Shoaff et al. 2016). These different results might be attributed to the number of urine samples from each woman. While Botton and colleagues used one spot urine sample at 26 mean gestational weeks from each study subject, Shoaff and his colleagues used two spot urine samples at approximately 16 and 26 weeks of gestation.

Impacts of high maternal concentrations of phenols and phthalates on birth weight have been reported. DEHP metabolites are positively associated with birth

weight and birth weight z-scores (Zhu et al. 2018). Meanwhile, BPA in utero exposures is associated with reduced birth weight and head circumference (Mustieles et al. 2018). Similar impacts were observed in neonates exposed to NP, where they presented with an increased risk of low neonatal weight (Chang et al. 2013). However, the effects of phenols seem to be short-lived because, at 4 years, children were reported with increased waist circumference (WC) and BMI (Valvi et al. 2013; Vafeiadi et al. 2016). Furthermore, phthalates have been implicated in other children's development indices. Boys who are between 20 and 36 months from high prenatal phthalate exposures had lower scores in language development (Olesen et al. 2018). In addition, benzyl butyl phthalate (BBP) and DEHP are associated with maternal reports of croup in infants up to 12 months of age (Shu et al. 2018).

3.3.2 Exposure to Persistent Chemicals

In human, most of the studies have addressed the effects of persistent chemicals on perinatal development. This review has focused on organochloride compounds (PCBs, DDT, DDE, and HCB), perfluoroalkyl substances (PFASs), dioxins and dioxin-like compounds, and polybrominated compounds (PBDEs and PBB).

Effects on Offspring's Growth and Neurodevelopment

Studies have examined the impact of early-life exposure to persistent chemicals on children's body and intellectual growth. Transplacental exposure to DDE has been associated with an increased height of boys at puberty, an effect which was not seen in lactationally exposed children (Gladen et al. 2000). This effect was not observed at infancy (Pan et al. 2010) or during the first year of life (Garced et al. 2012). Adolescent girls who were prenatally exposed to PFAS had reduced bone mass and size (Jeddy et al. 2018).

Studies have also examined the impacts of these chemicals on children's neurodevelopment. At birth, PBB-exposed infants are reported to have an increased odds of a below-median Apgar score at 1 and 5 min (Terrell et al. 2015). Later in life, effects are still being seen. High maternal serum levels of PBDE-47 at 16 weeks of gestation was associated with reduced cognitive abilities in 8 years children (Braun et al. 2017).

Young girls born to adolescent mothers with high PFAS serum levels had a lower vocabulary score at 15 months and lower language score at 38 months (Jeddy et al. 2017). This defect seems to disappear as the girls develop because there seems not to be any association between intelligent quotient (IQ) and PFAS exposure at 5 years (Liew et al. 2018).

Tai and colleagues observed that dioxins from breast milk, collected a month after birth, are associated with a decrease in expressive communication, composite, and gross motor scores in male offspring (Tai et al. 2016). This effect was not observed

in children exposed to DDE and DDT at 7 years of age (Jusko et al. 2012). However, worse psychomotor scores were recorded in 2 years children exposed prenatally to PCB-153 (Gascon et al. 2013). The sample size used in these studies to assess neurodevelopmental outcome was very small (range 217–1,175) with much diversity in socioeconomics, customs, cultures, and languages among study subjects. It is, therefore, difficult to generalize the findings. Studies with larger sample sizes are needed to confirm these neurodevelopmental outcomes.

Persistent chemicals are also attributed to early puberty onset. Breastfed girls born to mothers with high PBB serum levels had an earlier age at menarche and earlier pubic hair stage (Blanck et al. 2000). In addition, maternal DDE levels at 15 μg/L have been associated with a reduction of the age at menarche by 1 year (Vasiliu et al. 2004). This effect might be attributed to body weight as there was no significant association after adjusting for children's body weight.

Longnecker and his colleagues did a study about the effect of high maternal PCB serum levels on hearing abilities of 8-year-old offspring. The study reported a lack of association between maternal PCBs concentrations and sensorineural hearing (Longnecker et al. 2004).

Effects on Offspring's Reproductive Development

Perinatal exposure to persistent chemicals has been reported to affect offspring reproductive development by eliciting genital malformations, particularly, in male offspring. High breast milk levels of PBDEs and organochlorides increased prevalence of congenital cryptorchidism in boys (Damgaard et al. 2006; Main et al. 2007). PCBs in breast milk increased the incidence of undescended testes in exposed children (Brucker-Davis et al. 2008). Meanwhile, serum concentrations of PCBs and DDE were not associated with an increased incidence of cryptorchidism and hypospadias in children (Longnecker et al. 2002; Mcglynn et al. 2009). This might mean that, for persistent chemicals, breast milk chemical levels are good determinants of genital malformations than serum levels. The identified risk factor for hypospadias includes maternal exposure to EDCs through occupational, professional, and environmental exposure during fetal life (Kalfa et al. 2015). Other risk factors include maternal occupational exposure to EDCs, use of prescription drugs during the first trimester of pregnancy, maternal exposure to EDCs during early pregnancy, and the use of hair cosmetics at home (Haraux et al. 2017).

Other reproductive effects assessed includes fecundability and genital organs impairments. The effects on fecundity seem to differ by child's sex. In male offspring, PCBs and DDE maternal levels did not have any effect on semen quality (Vested et al. 2014). Meanwhile in female offspring, in utero exposure to PCBs from fish eaters decreased fecundity (Han et al. 2016). For the PCBs to pose a threat in human offspring reproductive health, women must be exposed to high doses. This is because contamination with low doses of PCBs does not pose a reproduction threat in humans (Khanjani and Sim 2007). Boys born to mothers with high PBDE-99 levels were reported to have reduced anogenital index (AGD/Weight)

(García-Villarino et al. 2018). This effect was not observed in boys born to mothers with higher serum DDE levels (Longnecker et al. 2007).

Effect on the Offspring's Weight and Other Metabolic Parameters

There is some evidence that portrays the role that endocrine disruptors play in obesity and the metabolic programming of obesity risk. EDCs that are known to induce obesity by regulating lipid metabolism and adipogenesis are referred to as obesogens (Hao et al. 2012). Serum or cord blood organochlorine levels are associated with impairment of offspring weight. A positive association has been reported between high HCB concentration in cord blood, as well as high maternal serum concentrations, with increased risk of overweight and obesity in children (Smink et al. 2008; Vafeiadi et al. 2015). Prenatal DDE and PCB exposure was observed to be associated with elevated BMI in young and adult girls (Karmaus et al. 2009; Mendez et al. 2011; Valvi et al. 2012), while for boys, overweight was observed to be associated with exposure to DDT (Valvi et al. 2012).

A similar effect has been observed about other persistent chemicals. Overweight was observed in children born to mothers with high PFAS serum concentrations (Lauritzen et al. 2018). Similarly, 7-year-old girls in a pooled cohort study from three European countries (Belgium, Norway, and Slovakia), born to mothers with high dioxin and dioxin-like compounds, were reported with an increased BMI (Iszatt et al. 2016).

Contrarily, a large sample size study done in the USA, which used third-trimester serum sample, did not find any association between exposures to persistent organochlorides and BMI among children followed up to 7 years (Cupul-Uicab et al. 2013). In addition, no effect on metabolic function was observed in mid-childhood children exposed in utero to PFAS (Fleisch et al. 2017). There is no exact reason that can explain the contradiction of results among studies; however, there is a significant variation of the sampling period among studies ranging from the first semester to the third trimester.

Effects on Offspring's Immune System

During early life, the human immune system, just as other systems, is vulnerable to immunotoxic EDCs. At 6 months of age, when children's immune system was tested on antibody responses in association with maternal serum PCBs, no specific antibody response was observed (Jusko et al. 2010). A study done in Japanese infants showed that, at about 10 months, dioxins, PCBs, and organochlorides significantly influence the percentages of lymphocyte subsets (Nagayama et al. 2007). At 18 months of age, children exposed prenatally to environmental levels of dioxins had increased risk of otitis media (Miyashita et al. 2011). At 24 months of life, fetal umbilical cord levels of some PFAS were associated with an increased risk of atopic dermatitis in female offspring (Chen et al. 2018).

In another study, there was a follow-up of the children from birth to 7 years. The study reported an association between maternal dioxin-like compounds concentrations and an increased frequency of wheezing in children aged up to 7 years (Miyashita et al. 2018). So far, the longest follow-up study was done for 20 years. This study, which was done in Denmark, yielded two conflicting reports. First, in 2014, they reported an increased risk of asthma in offspring exposed to PCB-118 and HCB (Hansen et al. 2014). Second, in 2016, they reported positive associations between in utero exposure to HCB, PCBs, and DDE and airway obstruction, but there was lack of association with allergic sensitization (Hansen et al. 2016). This study supports the understanding that chronic obstructive lung diseases are partly determined early in life and that the impact of early-life disadvantageous exposures persists beyond childhood (Svanes et al. 2010). However, it is important to note that, at 20 years follow-up, there is a high possibility for offspring to present adverse health effects associated with secondary exposure to environmental and dietary sources of EDCs.

Effects on Pregnancy Outcome

Many studies in this aspect focused and reported on the effects of organochloride chemicals. Among organochlorides, PCB in cord blood, maternal serum, and milk have been associated with reduced birth weight and head circumference (Hertz-Picciotto et al. 2005; Sagiv et al. 2007; Tan et al. 2009; Brucker-Davis et al. 2010). Reduced birth weight and head circumference in infants have also been associated with higher cord serum levels of HCB (Ribas-Fitó et al. 2002; Lauritzen et al. 2017). Birth weight is also negatively associated with DDT exposure (Sagiv et al. 2007). Other studies have reported lack of significant association between exposure to organochlorides and PBB during pregnancy and undesirable pregnancy outcomes that impair fetal growth (Gladen et al. 2003; Longnecker et al. 2005; Givens et al. 2007; Khanjani and Sim 2007; Sagiv et al. 2007; Wolff et al. 2007). Longer storage of the serum samples might have affected the stability of the chemicals and hence the observed lack of association between the parameters of interest.

Some researchers have investigated the association between organochlorides and congenital anomalies. In a case-control study done in China, serum organochlorides were measured in 117 mothers who delivered neural tube defect (NTD) infants (cases) and 121 mothers who delivered healthy infants (control). No association was observed between organochlorides exposure and the risk of NTDs (Wang et al. 2014). Meanwhile, Indian case-control study, which used only 35 mothers-neonates with NTDs (cases) and 35 mothers-neonate dyads without congenital anomalies (control), reported an association between NTDs and DDE (Kalra et al. 2016). The Indian study used both maternal and neonate serum; and both samples were positively associated with NTDs. However, more studies with larger sample sizes are needed to confirm these associations.

3.4 Mechanism of Action of EDCs

By definition, EDCs develop their effects by affecting the organism's endocrine system. The mechanisms by which the various EDCs disrupt the endocrine system differ. This difference is attributable to the variation in the susceptibility of the bodily tissues to each EDC, as well as the period by which each EDC disrupts the endocrine system. While some EDCs disrupt the endocrine system at the embryonic stage, others elicit their effects at the lactational stage. Generally, mechanisms involving elicitation of effects by endocrine disruptors involve two pathways: either directly on hormone-receptor complex or directly on the specific proteins that are involved in the control of delivery of hormones. EDCs act as agonists or antagonists of naturally occurring hormones, particularly, estrogens, androgens, and thyroid hormones. As agonists, they produce hormonal responses; and as antagonists, they inhibit hormonal responses (Kabir et al. 2015).

Estrogenic EDCs act by intracellularly or extracellularly binding to either classical, isoforms of classical, or nonclassical estrogen receptors (ERs) (ERα and ERβ) (Ropero et al. 2006). Intracellular estrogenic signal networks comprise the genomic pathway, which involves transcription of target genes, and the non-genomic pathway, which involves the rapid transduction of signals mediated by membrane-bound estrogen receptors (ERs) and/or other receptors, through crosstalk and/or bypassing. Extracellular estrogenic signal networks consist of pathways of autocrine and/or paracrine signaling, which involve other hormones, growth factors, and cytokines (Kiyama and Wada-Kiyama 2015). After binding to these receptors, estrogenic EDCs activate signaling cascades that, in many cases, finalize with the activation of transcription factors, turning a non-genomic response into a genomic one (Ropero et al. 2006).

Other EDCs act in an antiandrogenic mechanism where they disrupt the androgen-signaling pathway and influence androgen-sensitive tissues through androgen-receptor (AR) antagonism or steroid synthesis inhibition (Blystone et al. 2009). AR antagonists, like BPA and VZ, act by inhibiting the binding of androgens to AR, which leads to suppression of androgen-dependent gene expression (Sidorkiewicz et al. 2017).

Some EDCs are also known as thyroid disruptors (Patrick 2009). Offspring neurodevelopment can be affected when maternal thyroid hormone reaches the fetus and affect gene expression in the fetal brain (Zoeller and Crofton 2000). Some EDCs bind to thyroid transport proteins such as transthyretin and interfere with thyroid hormone transport resulting in either agonistic or antagonistic effects depending on the type of chemical and the target tissue. They can act as either thyroid receptor (TR) agonists and hence facilitate mRNA expression of well-known thyroid hormone response genes in the liver and in the pituitary or as thyroid receptor (TR) antagonists and hence inhibit TR-mediated gene activation of T3 (Ghassabian and Trasande 2018).

Obesogens, like TBT, TPT, and some phthalates, target transcription regulators (PPARα, δ, and γ) found in gene networks that function to control intracellular lipid

homeostasis as well as proliferation and differentiation of adipocytes (Yang et al. 2015). Transcription regulators are activated by heterodimerization with another receptor known as the 9-cis retinoic acid receptor. The process is regulated by the PPARγ, which associates with the RXR receptors and binds DNA targets as a heterodimer to directly regulate the expression at the transcriptional level (Grün and Blumberg 2009).

4 Conclusion

Results from studies show that in utero and lactational exposure to EDCs is associated with impairment of reproductive, immunologic, metabolic, neurobehavioral, and growth physiology of the exposed offspring up to the fourth generation. In some of the chemicals, a clear correlation exists between effects noted in the animal experimental and human epidemiological studies. However, little has been done in extrapolating animal toxicity studies into the human population. Based on the findings of this review, we would advocate that:

1. Future risk assessment for EDCs needs to take into account that humans are exposed to multiple chemicals that might share mechanisms and pathways in rendering their effects. Exposures to multiple chemicals necessitate the need for risk assessors to consider cumulative risks from each chemical.
2. Methodological approaches in both animal experiments and human epidemiological studies should be improved to achieve more specificity and better replication of results. The following should be considered:
   (a) Using multiple urine samples over the gestation period to better characterize ongoing exposure to nonpersistent EDCs.
   (b) Follow-up of perinatal exposure effects over several years should consider the confounding secondary exposure from environmental and dietary sources.
   (c) Animal experimental studies should move from the classical low throughput designs that have been used for decades into more advanced and precise designs.
3. Considering the ubiquitous daily exposure to persistent and nonpersistent EDCs across the lifespan, it is important to reassess the current safe chemical exposure levels and advocate for common-sense lifestyle changes in which females who wish to reproduce minimize their exposure to EDCs for the safety of future generations.
4. Further human studies are necessary to clarify the effects of perinatal exposure to EDCs as well as the physiologic mechanisms underlying these effects.

5 Summary

Animal experimental and human epidemiological studies reviewed have established an understanding that perinatal exposure to EDCs is associated with multiple adverse health effects in the exposed offspring. Little convergence is seen between animal experiments and human studies in terms of the reported effects. This might be due to methodological challenges as well as inadequate work that has been done in translating animal experiments by using high sample size human population studies. Since new chemicals, which have the potential of eliciting adverse health effects, are produced every day, it is very important to think of more sophisticated technologies with high throughput to screen-out unsafe chemicals.

Acknowledgments This work was supported by the National Key Research and Development Program of China (Grant No. 2018YFC1004401) and the National Natural Science Foundation of China (Grant No. 81671493).

Conflict of Interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

Acevedo N, Davis B, Schaeberle CM et al (2013) Perinatally administered bisphenol a as a potential mammary gland carcinogen in rats. Environ Health Perspect 121:1040–1046. https://doi.org/10.1289/ehp.1306734

Agatonovic-Kustrin S, Ling LH, Tham SY, Alany RG (2002) Molecular descriptors that influence the amount of drugs transfer into human breast milk. J Pharm Biomed Anal 29:103–119

Ahmad R, Gautam AK, Verma Y et al (2014) Effects of in utero di-butyl phthalate and butyl benzyl phthalate exposure on offspring development and male reproduction of rat. Environ Sci Pollut Res Int 21:3156–3165. https://doi.org/10.1007/s11356-013-2281-x

Alonso-Magdalena P, Vieira E, Soriano S et al (2010) Bisphenol a exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ Health Perspect 118:1243–1250. https://doi.org/10.1289/ehp.1001993

Altamirano GA, Muñoz-de-Toro M, Luque EH et al (2015) Milk lipid composition is modified by perinatal exposure to bisphenol A. Mol Cell Endocrinol 411:258–267. https://doi.org/10.1016/j.mce.2015.05.007

Andrade AJM, Grande SW, Talsness CE et al (2006) A dose response study following in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP): reproductive effects on adult male offspring rats. Toxicology 228:85–97. https://doi.org/10.1016/j.tox.2006.08.020

Angle BM, Do RP, Ponzi D et al (2013) Metabolic disruption in male mice due to fetal exposure to low but not high doses of bisphenol A (BPA): evidence for effects on body weight, food intake, adipocytes, leptin, adiponectin, insulin and glucose regulation. Reprod Toxicol 42:256–268. https://doi.org/10.1016/j.reprotox.2013.07.017

Anh T, Hori E, Pham TT et al (2013) Influence of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on socioemotional behaviors in offspring rats. Environ Health Insights 7:1–14. https://doi.org/10.4137/EHI.S10346

Anway MD, Memon MA, Uzumcu M, Skinner MK (2006) Transgenerational effect of the endocrine disruptor vinclozolin on male spermatogenesis. J Androl 27:868–879. https://doi.org/10.2164/jandrol.106.000349

Arai Y, Mori T, Suzuki Y, Bern HA (1983) Long-term effects of perinatal exposure to sex steroids and diethylstilbestrol on the reproductive system of male mammals. Int Rev Cytol 84:235–268

Arambula SE, Fuchs J, Cao J, Patisaul HB (2017) Effects of perinatal bisphenol A exposure on the volume of sexually-dimorphic nuclei of juvenile rats: a CLARITY-BPA consortium study. Neurotoxicology 63:33–42. https://doi.org/10.1016/j.neuro.2017.09.002

Aragon AC, Goens MB, Carbett E, Walker MK (2008) Perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure sensitizes offspring to angiotensin II-induced hypertension. Cardiovasc Toxicol 8:145–154. https://doi.org/10.1007/s12012-008-9023-1

Bansal A, Rashid C, Xin F et al (2017) Sex- and dose-specific effects of maternal bisphenol A exposure on pancreatic islets of first- and second-generation adult mice offspring. Environ Health Perspect 125:097022. https://doi.org/10.1289/EHP1674

Barakat R, Lin P-CP, Rattan S et al (2017) Prenatal exposure to DEHP induces premature reproductive senescence in male mice. Toxicol Sci 156:96–108. https://doi.org/10.1093/toxsci/kfw248

Barakat R, Lin P-C, Park CJ et al (2018) Prenatal exposure to DEHP induces neuronal degeneration and neurobehavioral abnormalities in adult male mice. Toxicol Sci 164:439–452. https://doi.org/10.1093/toxsci/kfy103

Bell DR, Clode S, Fan MQ et al (2007) Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin in the developing male Wistar(Han) rat. I: no decrease in epididymal sperm count after a single acute dose. Toxicol Sci 99:214–223. https://doi.org/10.1093/toxsci/kfm140

Bell MR, Dryden A, Will R, Gore AC (2018) Sex differences in effects of gestational polychlorinated biphenyl exposure on hypothalamic neuroimmune and neuromodulator systems in neonatal rats. Toxicol Appl Pharmacol 353:55–66. https://doi.org/10.1016/j.taap.2018.06.002

Blanck HM, Marcus M, Tolbert PE et al (2000) Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. Epidemiology 11:641–647. https://doi.org/10.1097/00001648-200011000-00005

Blystone CR, Lambright CS, Cardon MC et al (2009) Cumulative and antagonistic effects of a mixture of the antiandrogens vinclozolin and iprodione in the pubertal male rat. Toxicol Sci 111:179–188. https://doi.org/10.1093/toxsci/kfp137

Boberg J, Christiansen S, Axelstad M et al (2011) Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. Reprod Toxicol 31:200–209. https://doi.org/10.1016/j.reprotox.2010.11.001

Bodin J, Bølling AK, Becher R et al (2014) Transmaternal bisphenol A exposure accelerates diabetes type 1 development in NOD mice. Toxicol Sci 137:311–323. https://doi.org/10.1093/toxsci/kft242

Botton J, Philippat C, Calafat AM et al (2016) Phthalate pregnancy exposure and male offspring growth from the intra-uterine period to five years of age. Environ Res 151:601–609. https://doi.org/10.1016/j.envres.2016.08.033

Boudalia S, Berges R, Chabanet C et al (2014) A multi-generational study on low-dose BPA exposure in wistar rats: effects on maternal behavior, flavor intake and development. Neurotoxicol Teratol 41:16–26. https://doi.org/10.1016/j.ntt.2013.11.002

Bowers WJ, Nakai JS, Chu I et al (2004) Early developmental neurotoxicity of a PCB/organochlorine mixture in rodents after gestational and lactational exposure. Toxicol Sci 77:51–62. https://doi.org/10.1093/toxsci/kfg248

Braniste V, Jouault A, Gaultier E et al (2010) Impact of oral bisphenol A at reference doses on intestinal barrier function and sex differences after perinatal exposure in rats. PNAS 107:448–453. https://doi.org/10.1073/pnas.0907697107

Braun JM, Yolton K, Dietrich KN et al (2009) Prenatal bisphenol A exposure and early childhood behavior. Environ Health Perspect 117:1945–1952. https://doi.org/10.1289/ehp.0900979

Braun JM, Yolton K, Stacy SL et al (2017) Prenatal environmental chemical exposures and longitudinal patterns of child neurobehavior. Neurotoxicology 62:192–199. https://doi.org/10.1016/j.neuro.2017.07.027

Brown AS, Cheslack-Postava K, Rantakokko P et al (2018) Association of maternal insecticide Levels with autism in offspring from a National Birth Cohort. Am J psychiatry Adv. https://doi.org/10.1176/appi.ajp.2018.17101129

Brucker-Davis F, Wagner-Mahler K, Delattre I et al (2008) Cryptorchidism at birth in Nice area (France) is associated with higher prenatal exposure to PCBs and DDE, as assessed by colostrum concentrations. Hum Reprod 23:1708–1718

Brucker-Davis F, Wagner-Mahler K, Bornebusch L et al (2010) Exposure to selected endocrine disruptors and neonatal outcome of 86 healthy boys from Nice area (France). Chemosphere 81:169–176. https://doi.org/10.1016/j.chemosphere.2010.06.068

Bruner-Tran KL, Osteen KG (2011) Developmental exposure to TCDD reduces fertility and negatively affects pregnancy outcomes across multiple generations. Reprod Toxicol 31:344–350. https://doi.org/10.1016/j.reprotox.2010.10.003

Bushnell PJ, Moser VC, MacPhail RC et al (2002) Neurobehavioral assessments of rats perinatally exposed to a commercial mixture of polychlorinated biphenyls. Toxicol Sci 68:109–120

Cabaton NJ, Canlet C, Wadia PR et al (2013) Effects of low doses of bisphenol A on the metabolome of perinatally exposed CD-1 mice. Environ Health Perspect 121:586–593. https://doi.org/10.1289/ehp.1205588

Cagampang FR, Torrens C, Anthony FW, Hanson MA (2012) Developmental exposure to bisphenol A leads to cardiometabolic dysfunction in adult mouse offspring. J Dev Orig Health Dis 3:287–292. https://doi.org/10.1017/S2040174412000153

Carbone S, Samaniego YA, Cutrera R et al (2012) Different effects by sex on hypothalamic-pituitary axis of prepubertal offspring rats produced by in utero and lactational exposure to di-(2-ethylhexyl) phthalate (DEHP). Neurotoxicology 33:78–84. https://doi.org/10.1016/j.neuro.2011.11.009

Caspersen IH, Haugen M, Schjølberg S et al (2016) Maternal dietary exposure to dioxins and polychlorinated biphenyls (PCBs) is associated with language delay in 3 year old Norwegian children. Environ Int 91:180–187. https://doi.org/10.1016/j.envint.2016.02.031

Chamorro-Garcia R, Diaz-Castillo C, Shoucri BM et al (2012) Ancestral perinatal obesogen exposure results in a transgenerational thrifty phenotype in mice. Nat Commun 8:2012. https://doi.org/10.1038/s41467-017-01944-z

Chamorro-García R, Sahu M, Abbey RJ et al (2013) Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal exposure to the obesogen tributyltin in mice. Environ Health Perspect 121:359–366. https://doi.org/10.1289/ehp.1205701

Chang C, Chen M, Liao K et al (2013) The association between maternal nonylphenol exposure and parity on neonatal birth weight: a cohort study in Taiwan. Chemosphere 93:1145–1152. https://doi.org/10.1016/j.chemosphere.2013.06.048

Chang L-LL, Wun W-SSA, Wang PS (2014) Recovery from developmental nonylphenol exposure is possible for female rats. Chem Biol Interact 221:52–60. https://doi.org/10.1016/j.cbi.2014.07.010

Chen Q, Huang R, Hua L et al (2018) Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: a prospective birth cohort study. Environ Health 17:8. https://doi.org/10.1186/s12940-018-0352-7

Colciago A, Casati L, Mornati O et al (2009) Chronic treatment with polychlorinated biphenyls (PCB) during pregnancy and lactation in the rat part 2: effects on reproductive parameters, on sex behavior, on memory retention and on hypothalamic expression of aromatase and 5alpha-reductases in the offs. Toxicol Appl Pharmacol 239:46–54. https://doi.org/10.1016/j.taap.2009.04.023

Cupul-Uicab LA, Klebanoff MA, Brock JW, Longnecker MP (2013) Prenatal exposure to persistent organochlorines and childhood obesity in the U.S. collaborative perinatal project. Environ Health Perspect 121:1103–1109. https://doi.org/10.1289/ehp.1205901

Dai Y, Yang Y, Xu X, Hu Y (2015) Effects of uterine and lactational exposure to di-(2-ethylhexyl) phthalate on spatial memory and NMDA receptor of hippocampus in mice. Horm Behav 71:41–48. https://doi.org/10.1016/j.yhbeh.2015.03.008

Damgaard IN, Skakkebæk NE, Toppari J et al (2006) Persistent pesticides in human breast milk and cryptorchidism. Environ Health Perspect 114:1133–1138. https://doi.org/10.1289/ehp.8741

DeBartolo D, Jayatilaka S, Yan Siu N et al (2016) Perinatal exposure to benzyl butyl phthalate induces alterations in neuronal development/maturation protein expression, estrogen responses, and fear conditioning in rodents. Behav Pharmacol 27:77–82. https://doi.org/10.1097/FBP.0000000000000190

Desforges J-PW, Ross PS, Loseto LL (2012) Transplacental transfer of polychlorinated biphenyls and polybrominated diphenyl ethers in arctic beluga whales (Delphinapterus leucas). Environ Toxicol Chem 31:296–300. https://doi.org/10.1002/etc.750

Dessì-Fulgheri F, Porrini S, Farabollini F (2002) Effects of perinatal exposure to bisphenol A on play behavior of female and male juvenile rats. Environ Health Perspect 110(Suppl 3):403–407. https://doi.org/10.1289/ehp.110-1241190

Dorman DC, Chiu W, Hales BF et al (2018) Systematic reviews and meta-analyses of human and animal evidence of prenatal diethylhexyl phthalate exposure and changes in male anogenital distance. J Toxicol Environ Health B 21:207–226. https://doi.org/10.1080/10937404.2018.1505354

Doyle TJ, Bowman JL, Windell VL et al (2013) Transgenerational effects of di-(2-ethylhexyl) phthalate on testicular germ cell associations and spermatogonial stem cells in mice. Biol Reprod 88:112. https://doi.org/10.1095/biolreprod.112.106104

Dunder L, Halin Lejonklou M, Lind L et al (2018) Low-dose developmental bisphenol A exposure alters fatty acid metabolism in Fischer 344 rat offspring. Environ Res 166:117–129. https://doi.org/10.1016/j.envres.2018.05.023

Ema M, Miyawaki E (2001) Adverse effects on development of the reproductive system in male offspring of rats given monobutyl phthalate, a metabolite of dibutyl phthalate, during late pregnancy. Reprod Toxicol 15:189–194

Ema M, Miyawaki E (2002) Effects on development of the reproductive system in male offspring of rats given butyl benzyl phthalate during late pregnancy. Reprod Toxicol 16:71–76

Endo T, Kakeyama M, Uemura Y et al (2012) Executive function deficits and social-behavioral abnormality in mice exposed to a low dose of dioxin in utero and via lactation. PLoS One 7:e50741. https://doi.org/10.1371/journal.pone.0050741

Errico S, Nicolucci C, Migliaccio M et al (2017) Analysis and occurrence of some phenol endocrine disruptors in two marine sites of the northern coast of Sicily (Italy). Mar Pollut Bull 120 (1–2):68–74. https://doi.org/10.1016/j.marpolbul.2017.04.061

Fenton SE, Hamm JT, Birnbaum LS, Youngblood GL (2002) Persistent abnormalities in the rat mammary gland following gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Toxicol Sci 67:63–74

Fielden MR, Halgren RG, Tashiro CH et al (2001) Effects of gestational and lactational exposure to Aroclor 1242 on sperm quality and in vitro fertility in early adult and middle-aged mice. Reprod Toxicol 15:281–292

Fleisch AF, Rifas-Shiman SL, Mora AM et al (2017) Early-life exposure to perfluoroalkyl substances and childhood metabolic function. Environ Health Perspect 125:481–487. https://doi.org/10.1289/EHP303

Galyon KD, Farshidi F, Han G et al (2017) Maternal bisphenol A exposure alters rat offspring hepatic and skeletal muscle insulin signaling protein abundance. Am J Obstet Gynecol 216:290.e1–290.e9. https://doi.org/10.1016/j.ajog.2016.08.041

Garced S, Torre-Sanchez L, Cebria ME et al (2012) Prenatal dichlorodiphenyldichloroethylene (DDE) exposure and child growth during the first year of life. Environ Res 113:58–62. https://doi.org/10.1016/j.envres.2011.12.002

García-Villarino M, Riaño-Galán I, Rodriguez-Dehli AC et al (2018) Prenatal exposure to persistent organic pollutants and anogenital distance in children at 18 months. Horm Res Paediatr 90:1–7. https://doi.org/10.1159/000492236

Gascon M, Verner M-A, Guxensa M et al (2013) Evaluating the neurotoxic effects of lactational exposure to persistent organic pollutants (POPs) in Spanish children. Neurotoxicology 34:9–15. https://doi.org/10.1016/j.neuro.2012.10.006

Ghassabian A, Trasande L (2018) Disruption in thyroid signaling pathway: a mechanism for the effect of endocrine-disrupting chemicals on child neurodevelopment. Front Endocrinol (Lausanne) 9:204. https://doi.org/10.3389/fendo.2018.00204

Gillette R, Reilly MP, Topper VY et al (2017) Anxiety-like behaviors in adulthood are altered in male but not female rats exposed to low dosages of polychlorinated biphenyls in utero. Horm Behav 87:8–15. https://doi.org/10.1016/j.yhbeh.2016.10.011

Givens ML, Small CM, Terrell ML et al (2007) Maternal exposure to polybrominated and polychlorinated biphenyls: infant birth weight and gestational age. Chemosphere 69:1295–1304

Gladen BC, Ragan NB, Rogan WJ (2000) Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. J Pediatr 136:490–496. https://doi.org/10.1067/mpd.2000.103505

Gladen BC, Shkiryak-Nyzhnyk ZA, Chyslovska N et al (2003) Persistent organochlorine compounds and birth weight. Ann Epidemiol 13:151–157

Gore AC, Crews D, Doan LL et al (2014) Introduction to endocrine disrupting chemicals (EDCs): a guide for public interest organizations and policy-makers. Endocrine Society, Washington, p 76

Gray LE, Ostby J, Furr J et al (2000) Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat. Toxicol Sci 58:350–365. https://doi.org/10.1093/toxsci/58.2.350

Griffiths SK, Campbell JP (2015) Placental structure, function and drug transfer. Contin Educ Anaesth Crit Care Pain 15:84–89. https://doi.org/10.1093/bjaceaccp/mku013

Grote K, Hobler C, Andrade AJM et al (2007) Effects of in utero and lactational exposure to triphenyltin chloride on pregnancy outcome and postnatal development in rat offspring. Toxoicology 238:177–185. https://doi.org/10.1016/j.tox.2007.05.033

Grün F, Blumberg B (2009) Minireview: the case for obesogens. Mol Endocrinol 23:1127–1134. https://doi.org/10.1210/me.2008-0485

Gu H, Liu Y, Wang W et al (2016) In utero exposure to di-(2-ethylhexyl) phthalate induces metabolic disorder and increases fat accumulation in visceral depots of C57BL/6J mice offspring. Exp Ther Med 12:3806–3812. https://doi.org/10.3892/etm.2016.3820

Guerra MT, Scarano WR, de Toledo FC et al (2010) Reproductive development and function of female rats exposed to di-η-butyl-phthalate (DBP) in utero and during lactation. Reprod Toxicol 29:99–105. https://doi.org/10.1016/j.reprotox.2009.10.005

Han L, Hsu W-W, Todem D et al (2016) In utero exposure to polychlorinated biphenyls is associated with decreased fecundability in daughters of Michigan female fish eaters: a cohort study. Environ Health 15:92. https://doi.org/10.1186/s12940-016-0175-3

Hansen S, Strøm M, Olsen SF et al (2014) Maternal concentrations of persistent organochlorine pollutants and the risk of asthma in offspring: results from a prospective cohort with 20 years of follow-up. Environ Health Perspect 122:93–99. https://doi.org/10.1289/ehp.1206397

Hansen S, Strøm M, Olsen SF et al (2016) Prenatal exposure to persistent organic pollutants and offspring allergic sensitization and lung function at 20 years of age. Clin Exp Allergy 46:329–336. https://doi.org/10.1111/cea.12631

Hamm JT, Sparrow BR, Wolf D, Birnbaum LS (2000) In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin alters postnatal development of seminal vesicle epithelium. Toxicol Sci 54:424–430

Hao C, Cheng X, Xia H, Ma X (2012) The endocrine disruptor mono-(2-ethylhexyl) phthalate promotes adipocyte differentiation and induces obesity in mice. Biosci Rep 629:619–629. https://doi.org/10.1042/BSR20120042

Haraux E, Braun K, Buisson P et al (2017) Maternal exposure to domestic hair cosmetics and occupational endocrine disruptors is associated with a higher risk of hypospadias in the offspring. Int J Environ Res Public Health 14:1–14. https://doi.org/10.3390/ijerph14010027

Hass U, Christiansen S, Boberg J et al (2016) Low-dose effect of developmental bisphenol A exposure on sperm count and behaviour in rats. Andrology 4:594–607. https://doi.org/10.1111/andr.12176

Haavisto TE, Myllymäki SA, Adamsson NA et al (2006) The effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on testicular steroidogenesis in infantile male rats. Int J Androl 29:313–322. https://doi.org/10.1111/j.1365-2605.2005.00568.x

Hertz-Picciotto I, Charles MJ, James RA et al (2005) In utero polychlorinated biphenyl exposures in relation to fetal and early childhood growth. Epidemiology 16:648–656. https://doi.org/10.1097/01.ede.0000173043.85834.f3

Hipwell AE, Kahn LG, Factor-Litvak P et al (2019) Exposure to non-persistent chemicals in consumer products and fecundability: a systematic review. Hum Reprod Update 25:51–71. https://doi.org/10.1093/humupd/dmy032

Hojo R, Kakeyama M, Kurokawa Y et al (2008) Learning behavior in rat offspring after in utero and lactational exposure to either TCDD or PCB126. Environ Health Prev Med 13:169–180. https://doi.org/10.1007/s12199-008-0026-0

Honma S, Suzuki A, Buchanan DL et al (2002) Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. Reprod Toxicol 16:117–122

Ichihara T, Yoshino H, Imai N et al (2003) Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol A in rats. J Toxicol Sci 28:165–171

Ikeda M, Mitsui T, Setani K et al (2005) In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin in rats disrupts brain sexual differentiation. Toxicol Appl Pharmacol 205:98–105. https://doi.org/10.1016/j.taap.2004.09.010

Iszatt N, Stigum H, Govarts E et al (2016) Perinatal exposure to dioxins and dioxin-like compounds and infant growth and body mass index at seven years: a pooled analysis of three European birth cohorts. Environ Int 94:399–407. https://doi.org/10.1016/j.envint.2016.04.040

Jang YJ, Park HR, Kim TH et al (2012) High dose bisphenol A impairs hippocampal neurogenesis in female mice across generations. Toxicology 296:73–82. https://doi.org/10.1016/j.tox.2012.03.007

Jašarević E, Williams SA, Vandas GM et al (2013) Sex and dose-dependent effects of developmental exposure to bisphenol A on anxiety and spatial learning in deer mice (Peromyscus maniculatus bairdii) offspring. Horm Behav 63:180–189. https://doi.org/10.1016/j.yhbeh.2012.09.009

Jarrell JF, Gocmen A, Akyol D, Brant R (2002) Hexachlorobenzene exposure and the proportion of male births in Turkey 1935-1990. Reprod Toxicol 16:65–70

Jeddy Z, Hartman TJ, Taylor EV et al (2017) Prenatal concentrations of Perfluoroalkyl substances and early communication development in British girls. Early Hum Dev 109:15–20. https://doi.org/10.1016/j.earlhumdev.2017.04.004

Jeddy Z, Tobias JH, Taylor EV et al (2018) Prenatal concentrations of Perfluoroalkyl substances and bone health in British girls at age 17. Arch Osteoporos 13:84. https://doi.org/10.1007/s11657-018-0498-5

Jensen TK, Frederiksen H, Kyhl HB et al (2015) Prenatal exposure to phthalates and anogenital distance in male infants from a low-exposed Danish cohort (2010–2012). Environ Health Perspect 124:1107–1113. https://doi.org/10.1289/ehp.1509870

Jiang Y, Xia W, Zhu Y et al (2014) Mitochondrial dysfunction in early life resulted from perinatal bisphenol A exposure contributes to hepatic steatosis in rat offspring. Toxicol Lett 228:85–92. https://doi.org/10.1016/j.toxlet.2014.04.013

Jin MH, Ko HK, Hong CH, Han SW (2008) In utero exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin affects the development of reproductive system in mouse. Yonsei Med J 49:843–850. https://doi.org/10.3349/ymj.2008.49.5.843

Jones BA, Shimell JJ, Watson NV (2011) Pre- and postnatal bisphenol A treatment results in persistent deficits in the sexual behavior of male rats, but not female rats, in adulthood. Horm Behav 59:246–251. https://doi.org/10.1016/j.yhbeh.2010.12.006

Jusko TA, De Roos AJ, Schwartz SM et al (2010) A cohort study of developmental polychlorinated biphenyl (PCB) exposure in relation to post-vaccination antibody response at 6-months of age. Environ Res 110:388–395. https://doi.org/10.1016/j.envres.2010.02.010

Jusko TA, Klebanoff MA, Brock JW, Longnecker MP (2012) In-utero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and school-aged children. Epidemiology 23:689–698. https://doi.org/10.1097/EDE.0b013e31825fb61d

Kabir ER, Rahman MS, Rahman I (2015) A review on endocrine disruptors and their possible impacts on human health. Environ Toxicol Pharmacol 40:241–258. https://doi.org/10.1016/j.etap.2015.06.009

Kakeyama M, Sone H, Tohyama C (2008) Perinatal exposure of female rats to 2,3,7,8-tetrachlorodibenzo-p-dioxin induces central precocious puberty in the offspring. J Endocrinol 197:351–358. https://doi.org/10.1677/JOE-08-0062

Kakeyama M, Endo T, Zhang Y et al (2014) Disruption of paired – associate learning in rat offspring perinatally exposed to dioxins. Arch Toxicol 88:789–798. https://doi.org/10.1007/s00204-013-1161-y

Kalfa N, Haddad M, Guys J et al (2015) Is hypospadias associated with prenatal exposure to endocrine disruptors? A French collaborative controlled study of a cohort of 300 consecutive children without genetic defect. Eur Urol 68:1023–1030. https://doi.org/10.1016/j.eururo.2015.05.008

Kalra S, Dewan P, Batra P et al (2016) Organochlorine pesticide exposure in mothers and neural tube defects in offsprings. Reprod Toxicol 66:56–60. https://doi.org/10.1016/j.reprotox.2016.09.005

Karmaus W, Osuch J, Eneli I et al (2009) Maternal levels of dichlorodiphenyl-dichloroethylene (DDE) may increase weight and body mass index in adult female offspring. Occup Environ Med 66:143–149. https://doi.org/10.1136/oem.2008.041921

Kawai K, Nozaki T, Nishikata H et al (2003) Aggressive behavior and serum testosterone concentration during the maturation process of male mice: the effects of fetal exposure to bisphenol A. Environ Health Perspect 111:175–178. https://doi.org/10.1289/ehp.5440

Kaya H, Hany J, Fastabend A et al (2002) Effects of maternal exposure to a reconstituted mixture of polychlorinated biphenyls on sex-dependent behaviors and steroid hormone concentrations in rats: dose-response relationship. Toxicol Appl Pharmacol 178:71–81. https://doi.org/10.1006/taap.2001.9318

Khanjani N, Sim MR (2007) Maternal contamination with PCBs and reproductive outcomes in an Australian population. J Expo Sci Environ Epidemiol 17:191–195. https://doi.org/10.1038/sj.jes.7500495

Kim TS, Jung KK, Kim SS et al (2010) Effects of in utero exposure to DI(*n*-butyl) phthalate on development of male reproductive tracts in Sprague-Dawley rats. J Toxicol Environ Health A 73:1544–1559. https://doi.org/10.1080/15287394.2010.511579

Kim S, Eom S, Kim H-J et al (2018) Association between maternal exposure to major phthalates, heavy metals, and persistent organic pollutants, and the neurodevelopmental performances of their children at 1 to 2 years of age-CHECK cohort study. Sci Total Environ 624:377–384. https://doi.org/10.1016/j.scitotenv.2017.12.058

Kimura N, Kimura T, Suzuki M, Totsukawa K (2006) Effect of gestational exposure to nonylphenol on the development and fertility of mouse offspring. J Reprod Dev 52:789–795. https://doi.org/10.1274/0916-7625-25.4.272

Kiyama R, Wada-Kiyama Y (2015) Estrogenic endocrine disruptors: molecular mechanisms of action. Environ Int 83:11–40. https://doi.org/10.1016/j.envint.2015.05.012

Kobayashi K, Miyagawa M, Wang R-S et al (2002) Effects of in utero and lactational exposure to bisphenol A on somatic growth and anogenital distance in F1 rat offspring. Ind Health 40:375–381

Kobayashi K, Miyagawa M, Wang R et al (2009) Effects of in utero exposure to 2,2′,4,4′,5,5′-hexachlorobiphenyl on postnatal development and thyroid function in rat offspring. Ind Health 47:189–197

Kodavanti PRS, Coburn CG, Moser VC et al (2010) Developmental exposure to a commercial PBDE mixture, DE-71: neurobehavioral, hormonal, and reproductive effects. Toxicol Sci 116:297–312. https://doi.org/10.1093/toxsci/kfq105

Kuriyama SN, Chahoud I (2004) In utero exposure to low-dose 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) impairs male fertility and alters neurobehavior in rat offspring. Toxicology 202:185–197. https://doi.org/10.1016/j.tox.2004.05.006

Kuwahara R, Kawaguchi S, Kohara Y et al (2013) Perinatal exposure to low-dose bisphenol A impairs spatial learning and memory in male rats. J Pharmacol Sci 123:132–139. https://doi.org/10.1254/jphs.13093FP

Kwon S, Stedman DB, Elswick BA et al (2000) Pubertal development and reproductive functions of Crl:CD BR Sprague-Dawley rats exposed to bisphenol A during prenatal and postnatal development. Toxicol Sci 55:399–406

La MM, Harper R, Birnbaum LS et al (2010) Maternal dioxin exposure combined with a diet high in fat increases mammary cancer incidence in mice. Environ Health Perspect 118:596–601. https://doi.org/10.1289/ehp.0901047

La Merrill M, Karey E, Moshier E et al (2014) Perinatal exposure of mice to the pesticide DDT impairs energy expenditure and metabolism in adult female offspring. PLoS One 9:e103337. https://doi.org/10.1371/journal.pone.0103337

LaRocca J, Boyajian A, Brown C et al (2011) Effects of in utero exposure to bisphenol A or diethylstilbestrol on the adult male reproductive system. Birth Defects Res B Dev Reprod Toxicol 92:526–533. https://doi.org/10.1002/bdrb.20336

Lauritzen HB, Larose TL, Øien T et al (2017) Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case-cohort study. Pediatr Res 81:33–42. https://doi.org/10.1038/pr.2016.187

Lauritzen HB, Larose TL, Øien T et al (2018) Prenatal exposure to persistent organic pollutants and child overweight/obesity at 5-year follow-up: a prospective cohort study. Environ Health 17:9. https://doi.org/10.1186/s12940-017-0338-x

Lee K-I, Chiang C-W, Lin H-C et al (2016) Maternal exposure to di-(2-ethylhexyl) phthalate exposure deregulates blood pressure, adiposity, cholesterol metabolism and social interaction in mouse offspring. Arch Toxicol 90:1211–1224. https://doi.org/10.1007/s00204-015-1539-0

Lejonklou MH, Christiansen S, Örberg J et al (2016) Low-dose developmental exposure to bisphenol A alters the femoral bone geometry in wistar rats. Chemosphere 164:339–346. https://doi.org/10.1016/j.chemosphere.2016.08.114

Li M, Qiu L, Zhang Y et al (2013a) Dose-related effect by maternal exposure to di-(2-ethylhexyl) phthalate plasticizer on inducing hypospadiac male rats. Environ Toxicol Pharmacol 35:55–60. https://doi.org/10.1016/j.etap.2012.10.006

Li X-J, Jiang L, Chen L et al (2013b) Neurotoxicity of dibutyl phthalate in brain development following perinatal exposure: a study in rats. Environ Toxicol Pharmacol 36:392–402. https://doi.org/10.1016/j.etap.2013.05.001

Li G, Chang H, Xia W et al (2014) F0 maternal BPA exposure induced glucose intolerance of F2 generation through DNA methylation change in Gck. Toxicol Lett 228:192–199. https://doi.org/10.1016/j.toxlet.2014.04.012

Liew Z, Ritz B, Bach CC et al (2018) Prenatal exposure to perfluoroalkyl substances and IQ scores at age 5; a study in the Danish national birth cohort. Environ Health Perspect 126:067004. https://doi.org/10.1289/EHP2754

Lilienthal H, Hack A, Roth-Härer A et al (2006) Effects of developmental exposure to 2,2′,4,4′,5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. Environ Health Perspect 114:194–201. https://doi.org/10.1289/ehp.8391

Lim Y-H, Bae S, Kim B-N et al (2017) Prenatal and postnatal bisphenol A exposure and social impairment in 4-year-old children. Environ Health 16:79. https://doi.org/10.1186/s12940-017-0289-2

Lin Y, Wei J, Li Y et al (2011) Developmental exposure to di(2-ethylhexyl) phthalate impairs endocrine pancreas and leads to long-term adverse effects on glucose homeostasis in the rat. Am J Physiol Metab 301:E527–E538. https://doi.org/10.1152/ajpendo.00233.2011

Lind T, Lejonklou MH, Dunder L et al (2017) Low-dose developmental exposure to bisphenol A induces sex-specific effects in bone of Fischer 344 rat offspring. Environ Res 159:61–68. https://doi.org/10.1016/j.envres.2017.07.020

Longnecker MP, Klebanoff MA, Zhou H, Brock JW (2001) Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 358:110–114

Longnecker MP, Klebanoff MA, Brock JW et al (2002) Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. Am J Epidemiol 155:313–322

Longnecker MP, Hoffman HJ, Klebanoff MA et al (2004) In utero exposure to polychlorinated biphenyls and sensorineural hearing loss in 8-year-old children. Neurotoxicol Teratol 26:629–637. https://doi.org/10.1016/j.ntt.2004.04.007

Longnecker MP, Klebanoff MA, Brock JW, Guo X (2005) Maternal levels of polychlorinated biphenyls in relation to preterm and small-for-gestational-age birth. Epidemiology 16:641–647. https://doi.org/10.1097/01.ede.0000172137.45662.85

Longnecker MP, Gladen BC, Cupul-Uicab LA et al (2007) In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene (DDE) in relation to anogenital distance in male newborns from Chiapas, México. Am J Epidemiol 165:1015–1022. https://doi.org/10.1093/aje/kwk109

Ma T, Yin X, Han R et al (2017) Effects of in utero exposure to Di-n-Butyl Phthalate on testicular development in rat. Int J Environ Res Public Health 14:1284. https://doi.org/10.3390/ijerph14101284

Main KM, Kiviranta H, Virtanen HE et al (2007) Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environ Health Perspect 115:1519–1526. https://doi.org/10.1289/ehp.9924

Makita Y, Omura M, Ogata R (2004) Effects of perinatal simultaneous exposure to tributyltin (TBT) and p,p'-DDE (1,1-dichloro-2,2-bis(p-chlorophenyl) ethylene) on male offspring of Wistar rats. J Toxicol Environ Health A 67:385–395. https://doi.org/10.1080/15287390490273451

Mandrup K, Boberg J, Isling LK et al (2016) Low-dose effects of bisphenol A on mammary gland development in rats. Andrology 4:673–683. https://doi.org/10.1111/andr.12193

Martinez-Arguelles DB, McIntosh M, Rohlicek CV et al (2013) Maternal in utero exposure to the endocrine disruptor di-(2-ethylhexyl) phthalate affects the blood pressure of adult male offspring. Toxicol Appl Pharmacol 266:95–100. https://doi.org/10.1016/j.taap.2012.10.027

Mcglynn KA, Guo X, Graubard BI et al (2009) Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. Environ Health Perspect 117:1472–1476. https://doi.org/10.1289/ehp.0800389

Mendez MA, Garcia-Esteban R, Guxens M et al (2011) Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect 119:272–278. https://doi.org/10.1289/ehp.1002169

Mendiola J, Stahlhut RW, Jørgensen N et al (2011) Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. Environ Health Perspect 119:958–963. https://doi.org/10.1289/ehp.1103421

Mennigen JA, Thompson LM, Bell M et al (2018) Transgenerational effects of polychlorinated biphenyls: 1. Development and physiology across 3 generations of rats. Environ Health 17:18. https://doi.org/10.1186/s12940-018-0362-5

Mira-Escolano MP, Mendiola J, Mínguez-Alarcón L et al (2014) Anogenital distance of women in relation to their mother's gynaecological characteristics before or during pregnancy. Reprod Biomed Online 28:209–215. https://doi.org/10.1016/j.rbmo.2013.09.026

Miyashita C, Sasaki S, Saijo Y et al (2011) Effects of prenatal exposure to dioxin-like compounds on allergies and infections during infancy. Environ Res 111:551–558. https://doi.org/10.1016/j.envres.2011.01.021

Miyashita C, Bamai YA, Araki A et al (2018) Prenatal exposure to dioxin-like compounds is associated with decreased cord blood IgE and increased risk of wheezing in children aged up to 7 years: the Hokkaido study. Sci Total Environ 610–611:191–199. https://doi.org/10.1016/j.scitotenv.2017.07.248

Moher D, Liberati A, Tetzlaff J et al (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 6:e1000097. https://doi.org/10.1371/journal.pmed1000097

Moon HJ, Han SY, Shin J-H et al (2007) Gestational exposure to nonylphenol causes precocious mammary gland development in female rat offspring. J Reprod Dev 53:333–344

Moore RW, Rudy TA, Lin TM et al (2001) Abnormalities of sexual development in male rats with in utero and lactational exposure to the antiandrogenic plasticizer Di(2-ethylhexyl) phthalate. Environ Health Perspect 109:229–237. https://doi.org/10.1289/ehp.01109229

Mustieles V, Williams PL, Fernandez MF et al (2018) Maternal and paternal preconception exposure to bisphenols and size at birth. Hum Reprod 33:1528–1537. https://doi.org/10.1093/humrep/dey234

Mylchreest E, Wallace DG, Cattley RC, Foster PM (2000) Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to Di(n-butyl) phthalate during late gestation. Toxicol Sci 55:143–151

Nagayama J, Tsuji H, Iida T et al (2007) Immunologic effects of perinatal exposure to dioxins, PCBs and organochlorine pesticides in Japanese infants. Chemosphere 67:S393–S398. https://doi.org/10.1016/j.chemosphere.2006.05.134

Nassr ACC, Arena AC, Toledo FC et al (2010) Effects of gestational and lactational fenvalerate exposure on immune and reproductive systems of male rats. J Toxicol Environ Health A 73:952–964. https://doi.org/10.1080/15287391003751745

Nguyen MN, Nishijo M, Nguyen ATNN et al (2013) Effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on parvalbumin- and calbindin-immunoreactive neurons in the limbic system and superior colliculus in rat offspring. Toxicology 314:125–134. https://doi.org/10.1016/j.tox.2013.09.005

Nilsson EE, Anway MD, Stanfield J, Skinner MK (2008) Transgenerational epigenetic effects of the endocrine disruptor vinclozolin on pregnancies and female adult onset disease. Reproduction 135:713–721. https://doi.org/10.1530/REP-07-0542

Nishijo M, Kuriwaki J-I, Hori E et al (2007) Effects of maternal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin on fetal brain growth and motor and behavioral development in offspring rats. Toxicol Lett 173:41–47. https://doi.org/10.1016/j.toxlet.2007.06.007

Ohsako S, Miyabara Y, Sakaue M et al (2002) Developmental stage-specific effects of perinatal 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure on reproductive organs of male rat offspring. Toxicol Sci 66:283–292

Olesen TS, Bleses D, Andersen HR et al (2018) Prenatal phthalate exposure and language development in toddlers from the Odense child cohort. Neurotoxicol Teratol 65:34–41. https://doi.org/10.1016/j.ntt.2017.11.004

Orito K, Gotanda N, Murakami M et al (2007) Prenatal exposure to 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) promotes anxiogenic behavior in rats. Tohoku J Exp Med 212:151–157

Pan I-J, Daniels JL, Herring AH et al (2010) Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant growth: an analysis of the pregnancy, infection, and nutrition babies study. Paediatr Perinat Epidemiol 24:262–271. https://doi.org/10.1111/j.1365-3016.2010.01114.x

Patrick L (2009) Thyroid disruption: mechanism and clinical implications in human health. Altern Med Rev 14:326–346

Phillips AL, Chen A, Rock KD et al (2016) Transplacental and lactational transfer of firemaster® 550 components in dosed wistar rats. Toxicol Sci 153:246–257. https://doi.org/10.1093/toxsci/kfw122

Pocar P, Fiandanese N, Berrini A et al (2017) Maternal exposure to di(2-ethylhexyl)phthalate (DEHP) promotes the transgenerational inheritance of adult-onset reproductive dysfunctions through the female germline in mice. Toxicol Appl Pharmacol 322:113–121. https://doi.org/10.1016/j.taap.2017.03.008

Pocock VJ, Sales GD, Wilson CA, Milligan SR (2002) Effects of perinatal octylphenol on ultrasound vocalization, behavior and reproductive physiology in rats. Physiol Behav 76:645–653. https://doi.org/10.1016/S0031-9384(02)00788-6

Ribas-Fitó N, Sala M, Cardo E et al (2002) Association of hexachlorobenzene and other organochlorine compounds with anthropometric measures at birth. Pediatr Res 52:163–167. https://doi.org/10.1023/01.PDR.0000023171.89966.8B

Ropero AB, Alonso-Magdalena P, Ripoll C et al (2006) Rapid endocrine disruption: environmental estrogen actions triggered outside the nucleus. J Steroid Biochem Mol Biol 102:163–169. https://doi.org/10.1016/j.jsbmb.2006.09.019

Rubin BS, Paranjpe M, Dafonte T et al (2016) Perinatal BPA exposure alters body weight and composition in a dose specific and sex specific manner: the addition of peripubertal exposure exacerbates adverse effects in female mice. Reprod Toxicol 68:130–144. https://doi.org/10.1016/j.reprotox.2016.07.020

Ryan BC, Hotchkiss AK, Crofton KM, Gray LE (2010) In utero and lactational exposure to bisphenol A, in contrast to ethinyl estradiol, does not alter sexually dimorphic behavior, puberty, fertility, and anatomy of female LE rats. Toxicol Sci 114:133–148. https://doi.org/10.1093/toxsci/kfp266

Sagiv SK, Tolbert PE, Altshul LM, Korrick SA (2007) Organochlorine exposures during pregnancy and infant size at birth. Epidemiology 18:120–129. https://doi.org/10.1097/01.ede.0000249769.15001.7c

Salian S, Doshi T, Vanage G (2009) Perinatal exposure of rats to bisphenol A affects the fertility of male offspring. Life Sci 85:742–752. https://doi.org/10.1016/j.lfs.2009.10.004

Sanabria M, Cucielo MS, Guerra MT et al (2016) Sperm quality and fertility in rats after prenatal exposure to low doses of TCDD: a three-generation study. Reprod Toxicol 65:29–38. https://doi.org/10.1016/j.reprotox.2016.06.019

Schneider S, Kaufmann W, Buesen R, van Ravenzwaay B (2008) Vinclozolin – the lack of a transgenerational effect after oral maternal exposure during organogenesis. Reprod Toxicol 25:352–360. https://doi.org/10.1016/j.reprotox.2008.04.001

Schönfelder G, Flick B, Mayr E et al (2002) In utero exposure to low doses of bisphenol A lead to long-term deleterious effects in the vagina. Neoplasia 4:98–102. https://doi.org/10.1038/sj/neo/7900212

Scott HM, Hutchison GR, Jobling MS et al (2008) Relationship between androgen action in the "male programming window," fetal sertoli cell number, and adult testis size in the rat. Endocrinology 149:5280–5287. https://doi.org/10.1210/en.2008-0413

Shin MY, Lee S, Choi H et al (2017) Placental and lactational transfer of decabromodiphenyl ether and 2,2′,4,4′-tetrabromodiphenyl ether in dam-offspring pairs of Sprague-Dawley rats. Food Chem Toxicol 102:198–203. https://doi.org/10.1016/j.fct.2017.01.027

Shoaff JR, Romano ME, Yolton K et al (2016) Prenatal phthalate exposure and infant size at birth and gestational duration. Environ Res 150:52–58. https://doi.org/10.1016/j.envres.2016.05.033

Shono T, Suita S, Kai H, Yamaguchi Y (2004) The effect of a prenatal androgen disruptor, vinclozolin, on gubernacular migration and testicular descent in rats. J Pediatr Surg 39:213–216

Shu H, Wikstrom S, Jönsson BAG et al (2018) Prenatal phthalate exposure was associated with croup in Swedish infants. Acta Paediatr 107:1011–1019. https://doi.org/10.1111/apa.14245

Si J, Han X, Zhang F et al (2012a) Perinatal exposure to low doses of tributyltin chloride advances puberty and affects patterns of estrous cyclicity in female mice. Environ Toxicol 27:662–670. https://doi.org/10.1002/tox.21756

Si J, Li J, Zhang F et al (2012b) Effects of perinatal exposure to low doses of tributyltin chloride on pregnancy outcome and postnatal development in mouse offspring. Environ Toxicol 27:605–612. https://doi.org/10.1002/tox.20753

Si J, Li P, Xin Q et al (2013) Perinatal exposure to low doses of tributyltin chloride reduces sperm count and quality in mice. Environ Toxicol 27:662–670. https://doi.org/10.1002/tox.21892

Sidorkiewicz I, Zareba K, Wołczynski S, Czerniecki J (2017) Endocrine-disrupting chemicals – mechanisms of action on male reproductive system. Toxicol Ind Health 33:601–609. https://doi.org/10.1177/0748233717695160

Smink A, Ribas-Fito N, Garcia R et al (2008) Exposure to hexachlorobenzene during pregnancy increases the risk of overweight in children aged 6 years. Acta Paediatr 97:1465–1469. https://doi.org/10.1111/j.1651-2227.2008.00937.x

Song S, Zhang L, Zhang H et al (2014) Perinatal BPA exposure induces hyperglycemia, oxidative stress and decreased adiponectin production in later life of male rat offspring. Int J Environ Res Public Health 11:3728–3742. https://doi.org/10.3390/ijerph110403728

Spörndly-Nees E, Boberg J, Ekstedt E et al (2018) Low-dose exposure to bisphenol A during development has limited effects on male reproduction in midpubertal and aging Fischer 344 rats. Reprod Toxicol 81:196–206. https://doi.org/10.1016/j.reprotox.2018.08.007

Steinberg RM, Walker DM, Juenger TE et al (2008) Effects of perinatal polychlorinated biphenyls on adult female rat reproduction: development, reproductive physiology, and second generational effects. Biol Reprod 78:1091–1101. https://doi.org/10.1095/biolreprod.107.067249

Stillman RJ (1982) In utero exposure to diethylstilbestrol: adverse effects on the reproductive tract and reproductive performance in male and female offspring. Am J Obstet Gynecol 142:905–921. https://doi.org/10.1016/S0002-9378(16)32540-6

Starling AP, Adgate JL, Hamman RF et al (2017) Perfluoroalkyl substances during pregnancy and offspring weight and adiposity at birth: examining mediation by maternal fasting glucose in the healthy start study. Environ Health Perspect 125:067016. https://doi.org/10.1289/EHP641

Stump DG, Beck MJ, Radovsky A et al (2010) Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats. Toxicol Sci 115:167–182. https://doi.org/10.1093/toxsci/kfq025

Sugawara N, Nakai K, Nakamura T et al (2006) Developmental and neurobehavioral effects of perinatal exposure to polychlorinated biphenyls in mice. Arch Toxicol 80:286–292. https://doi.org/10.1007/s00204-005-0042-4

Sun X, Li D, Liang H et al (2018) Maternal exposure to bisphenol A and anogenital distance throughout infancy: a longitudinal study from Shanghai, China. Environ Int 121:269–275. https://doi.org/10.1016/j.envint.2018.08.055

Susiarjo M, Xin F, Bansal A et al (2015) Bisphenol A exposure disrupts metabolic health across multiple generations in the mouse. Endocrinology 156:2049–2058. https://doi.org/10.1210/en.2014-2027

Svanes C, Sunyer J, Plana E et al (2010) Early life origins of chronic obstructive pulmonary disease. Thorax 65:14–20. https://doi.org/10.1136/thx.2008.112136

Swan SH, Main KM, Liu F et al (2005) Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect 113:1056–1061. https://doi.org/10.1289/ehp.8100

Takagi H, Shibutani M, Masutomi N et al (2004) Lack of maternal dietary exposure effects of bisphenol A and nonylphenol during the critical period for brain sexual differentiation on the reproductive/endocrine systems in later life. Arch Toxicol 78:97–105. https://doi.org/10.1007/s00204-003-0517-0

Tai PT, Nishijo M, Nghi TN et al (2016) Effects of perinatal dioxin exposure on development of children during the first 3 years of life. J Pediatr 175:159–166.e2. https://doi.org/10.1016/j.jpeds.2016.04.064

Tan J, Loganath A, Chong YS, Obbard JP (2009) Exposure to persistent organic pollutants in utero and related maternal characteristics on birth outcomes: a multivariate data analysis approach. Chemosphere 74:428–433. https://doi.org/10.1016/j.chemosphere.2008.09.045

Terrell ML, Hartnett KP, Lim H et al (2015) Maternal exposure to brominated flame retardants and infant Apgar scores. Chemosphere 118:178–186. https://doi.org/10.1016/j.chemosphere.2014.08.007

U.S. Environmental Protection Agency (1997) Special report on environmental endocrine disruption: an effects assessment and analysis. Risk Assess Forum Washington DC EPA/630/R

U.S. Environmental Protection Agency (2013) America's children and the environment, 3rd edn. US EPA, Washington

UNEP (2010) Stockholm convention on persistent organic pollutants. As amended in 2009

Vafeiadi M, Agramunt S, Papadopoulou E et al (2013) In utero exposure to dioxins and dioxin-like compounds and anogenital distance in newborns and infants. Environ Health Perspect 121:125–130. https://doi.org/10.1289/ehp.1205221

Vafeiadi M, Georgiou V, Chalkiadaki G et al (2015) Association of prenatal exposure to persistent organic pollutants with obesity and cardiometabolic traits in early childhood: the Rhea mother–child cohort (Crete, Greece). Environ Health Perspect 123:1015–1021. https://doi.org/10.1289/ehp.1409062

Vafeiadi M, Roumeliotaki T, Myridakis A et al (2016) Association of early life exposure to bisphenol A with obesity and cardiometabolic traits in childhood. Environ Res 146:379–387. https://doi.org/10.1016/j.envres.2016.01.017

Valvi D, Mendez MA, Martinez D et al (2012) Prenatal concentrations of polychlorinated biphenyls, DDE, and DDT and overweight in children: a prospective birth cohort study. Environ Health Perspect 120:451–457

Valvi D, Casas M, Mendez MA et al (2013) Prenatal bisphenol A urine concentrations and early rapid growth and overweight risk in the offspring. Epidemiology 24:791–799. https://doi.org/10.1097/EDE.0b013e3182a67822

Vasiliu O, Muttineni J, Karmaus W (2004) In utero exposure to organochlorines and age at menarche. Hum Reprod 19:1506–1512. https://doi.org/10.1093/humrep/deh292

Vested A, Ramlau-Hansen CH, Olsen SF et al (2014) In utero exposure to persistent organochlorine pollutants and reproductive health in the human male. Reproduction 148:635–646. https://doi.org/10.1530/REP-13-0488

Vreugdenhil HJI, Mulder PGH, Emmen HH, Weisglas-kuperus N (2004) Effects of perinatal exposure to PCBs on neuropsychological functions in the Rotterdam cohort at 9 years of age. Neuropsychology 18:185–193. https://doi.org/10.1037/0894-4105.18.1.185

Wang B, Yi D, Jin L et al (2014) Organochlorine pesticide levels in maternal serum and risk of neural tube defects in offspring in Shanxi Province, China: a case-control study. Sci Total Environ 490:1037–1043. https://doi.org/10.1016/j.scitotenv.2014.05.075

Wells GA, Shea B, O'Connell D et al (2013) The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa Hospital Research Institute, Ottawa. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 25 Feb 2019

Wolff MS, Engel S, Berkowitz G et al (2007) Prenatal pesticide and PCB exposures and birth outcomes. Pediatr Res 61:243–250. https://doi.org/10.1203/pdr.0b013e31802d77f0

Woodruff TJ, Zota AR, Schwartz JM (2011) Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environ Health Perspect 119:878–885. https://doi.org/10.1289/ehp.1002727

Xu X-H, Wang Y-M, Zhang J et al (2010) Perinatal exposure to bisphenol-A changes n-methyl-d-aspartate receptor expression in the hippocampus of male rat offspring. Environ Toxicol Chem 29:176–181. https://doi.org/10.1002/etc.18

Xu X, Yang Y, Wang R et al (2015) Perinatal exposure to di-(2-ethylhexyl) phthalate affects anxiety- and depression-like behaviors in mice. Chemosphere 124:22–31. https://doi.org/10.1016/j.chemosphere.2014.10.056

Yang O, Kim HL, Weon J-I, Seo YR (2015) Endocrine-disrupting chemicals: review of toxicological mechanisms using molecular pathway analysis. J Cancer Prev 20:12–24. https://doi.org/10.15430/JCP.2015.20.1.12

Yang J, Yu J, Wang P et al (2017) The adverse effects of perinatal exposure to nonylphenol on carbohydrate metabolism in male offspring rats. Int J Environ Health Res 27:368–376. https://doi.org/10.1080/09603123.2017.1373275

Zhu Y, Wan Y, Zhang B et al (2018) Relationship between maternal phthalate exposure and offspring size at birth. Sci Total Environ 612:1072–1078. https://doi.org/10.1016/j.scitotenv.2017.08.207

Zoeller RT, Crofton KM (2000) Thyroid hormone action in fetal brain development and potential for disruption by environmental chemicals. Neurotoxicology 21:935–945