Current Topics in
Environmental Health and Preventive Medicine

Reiko Kishi
Philippe Grandjean *Editors*

Health Impacts of Developmental Exposure to Environmental Chemicals

Springer

Current Topics in Environmental Health and Preventive Medicine

Series Editor

Takemi Otsuki
Kawasaki Medical School
Kurashiki
Okayama, Japan

Current Topics in Environmental Health and Preventive Medicine, published in partnership with the Japanese Society of Hygiene, is designed to deliver well written volumes authored by experts from around the globe, covering the prevention and environmental health related to medical, biological, molecular biological, genetic, physical, psychosocial, chemical, and other environmental factors. The series will be a valuable resource to both new and established researchers, as well as students who are seeking comprehensive information on environmental health and health promotion.

More information about this series at http://www.springer.com/series/13556

Reiko Kishi • Philippe Grandjean
Editors

Health Impacts of Developmental Exposure to Environmental Chemicals

Springer

Editors
Reiko Kishi
Center for Environmental and Health Sciences
Hokkaido University
Sapporo
Hokkaido
Japan

Philippe Grandjean
Department of Environmental Health
Harvard T.H. Chan School of Public Health
Boston, MA
USA

Department of Environmental Medicine
University of Southern Denmark
Odense
Denmark

ISSN 2364-8333 ISSN 2364-8341 (electronic)
Current Topics in Environmental Health and Preventive Medicine
ISBN 978-981-15-0519-5 ISBN 978-981-15-0520-1 (eBook)
https://doi.org/10.1007/978-981-15-0520-1

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

The concern about the environment and children's health is growing internationally, especially regarding the life-long health effects of prenatal exposures to chemical hazards. This book aims to cover a wide range of issues related to important adverse health risks and opportunities for the prevention of children's adverse health risks that may lead to early and future diseases. This book consists of five parts "to make everyone stand at a starting point for a healthy life."

Part I is Introduction (Chapter 1). It begins with the fetal Minamata disease. Severe neurodevelopmental disorders have been observed with remarkable differences in pathological finding between adults and children. The cause of fetal Minamata disease was methylmercury. We can learn the lesson from the history of 60 years ago.

Part II (Adverse Health Effects on Human Developing Organs Caused by Environmental Chemicals: The Role That Chemicals Might Play; Chaps. 2–9) consists of eight chapters that cover pregnancy abnormalities, decreased birth weight, birth defects, neurodevelopment, immune dysfunction, developmental programming and obesity, and reproductive hormones.

Part III (Impact of Environmental Chemical Hazards on Human Development; Chaps. 10–16) deals with the next-generation effects of environmental chemicals, including atmospheric particulate matter, trace metals, PCB/Dioxins, perfluorinated and brominated chemicals, phthalates, and bisphenols.

Part IV (Important Aspects of Research for Newly Developed Areas and Prevention Strategy; Chaps. 17–21) consists of five chapters: Adverse outcome pathways for developmental toxicity, exposomics, genetic susceptibility, epigenetics, and finally the interface between science and policy. Finally, Part V (Chap. 22) shows further direction of research and policy making of Environment and Children's Health. Many challenges in the future studies are pointed out for everyone's healthy life.

Overall, the 22 chapters, including introduction and overview chapters, outline the current state of children's environmental health, the significance of different hazards and research approaches, and the importance of evidence-based scientific policies in environmental health.

Both editors realized the importance of protecting the next generation against adverse effects when the natural history of the Minamata disease was uncovered. Reiko Kishi was a medical student when she first learned about the victims of methyl-mercury poisoning. Then she became a PhD candidate studying neurobehavioral toxicology, and she particularly desired to find the most sensitive methods to detect early subclinical dysfunction level rather than irreversible pathological disorders. After learning environmental epidemiology, she realized that several scientific fields relate to health impacts of developmental exposure and the importance of community level public health approaches. Likewise, when Philippe Grandjean was in medical school in 1972 he saw Minamata victims in a TV program on the United Nations environment conference in Stockholm. It made a deep impression, as his medical studies had not prepared him for disasters caused by industrial use of toxic chemicals. Both of the editors decided that the medical profession had to address the environmental pollution and aim at protecting the next generation against hazardous exposures.

As colleagues with parallel experiences and research studies of many thousands of children, we decided to collaborate on a textbook that would summarize the current knowledge on environmental hazards that can endanger the health of the next generation. Although testing chemicals for adverse effects during early development is not yet routine, experimental evidence lends substantiation to the enduring damage toxic chemicals exert if incurred during early development, though some may not be apparent right away. During recent years, medical research in this field has bourgeoned, in particular in the form of prospective studies of birth cohorts, like the ones in Hokkaido, Japan, and the Faroe Islands. Also, recently, substantial research efforts on birth cohorts have been initiated in Asia. It therefore felt natural to invite colleagues mainly from Asian countries to contribute to chapters of this book.

At a time when environmental pollution has grown into a global crisis, we realize that our generation has not properly managed the responsibility for a healthy environment and for the protection against environmentally mediated hazards, especially the ones that can cause harm during early development. Our ambition is that this book will help inspire and educate colleagues, heath care professionals, and parents to safeguard future generations. Furthermore, we hope that when carrying out risk communication in countries, people will correctly recognize the situation, understand difficult problems as familiar problems as close as possible, and are linked to global environmental policy backed by scientific evidence.

Sapporo, Japan — Reiko Kishi
Odense, Denmark — Philippe Grandjean

Acknowledgements

In the process of inviting chapter authors, communicating about format and space limitations, and adjusting manuscripts for production, we have received enthusiastic collaboration from the authors. Peer review was undertaken with the generous help of Atsuki Araki and Yufu Aitbamai (Hokkaido University) and Tina Kold Jensen (University of Southern Denmark).

As editors, we have depended on support from our close colleagues, both Mimi Takahashi and Noriko Tanaka (Hokkaido University) and Colleen Bouzan (Harvard University). We are extremely grateful for their highly qualified and generous support.

Contents

Part I
Introduction

Chapter 1
Impacts of Developmental Exposure to Environmental Chemicals on Human Health with Global Perspectives

Reiko Kishi

Abstract There is global concern about children's environment and health and the environmental effects of chemical exposure. This began with the fetal Minamata disease that occurred more than 60 years ago in Japan. Severe neurodevelopmental disorders and cerebral palsy-like symptoms have been observed with remarkable differences in pathological findings between adults and children. The mother's symptoms have been generally mild. The cause of fetal Minamata disease was methylmercury contained in factory effluent. Since the fetus has a high demand for neutral amino acids, methylmercury is actively transported transplacentally to the fetus. Moreover, it is highly sensitive because of the time window when cells develop most. In the same Kyusyu island, Yusho, (the Kanemi rice-bran oil poisoning) occurred in 1968. At the beginning of the incident, the cause of disease was explained due to PCB (polychlorinated biphenyls), but later dioxins such as PCDD, PCDF were detected in the blood of the patients. There are many crucial points we should learn from these historical disasters, including not only the scientific causalities but also the social and political backgrounds in economically rapid growth. The twenty-first century is the era of birth cohort studies throughout the world. In Asia, several birth cohorts were launched in early 2000. As of April 2019, 31 cohorts in 16 countries have joined the BiCCA (Birth Cohort Consortium in Asia) and they are ongoing with life-course DOHaD (developmental origin of health and disease) approaches. The integration of "environment" into the DOHaD theory became new paradigm especially for the prevention of disease/dysfunction focusing on both clinical and public health implications.

Keywords Fetal Minamata disease · Developmental disorder · Umbilical cord · Methylmercury · Rice-bran oil disease (Yusho) · POPs (Persistent Orgnic Pollutants) · Our stolen future · Birth cohorts · Life course approach · DOHaD

R. Kishi (✉)
Hokkaido University Center for Environmental and Health Sciences, Sapporo, Japan
e-mail: rkishi@med.hokudai.ac.jp

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_1

1.1 The Minamata Disaster

In April 1956, a 5-year-old girl from the city of Minamata with speech, gait, and intelligence problems entered the Chisso Chemical Plant affiliated hospital. The pediatrician Dr. Kaneyoshi Noda examined her and attempted to diagnose her condition but was unsuccessful and 1 week later her 3-year-old sister was hospitalized with similar symptoms. Their mother reported that there were many patients with similar symptoms in the area (Fig. 1.1). Dr. Hajime Hosokawa, the head of the hospital, reported this to the Minamata Public Health Center stating that "unexplained neurological disorders of children are occurring." This was the first case of an official discovery of Minamata disease. "Cerebral palsy" was detected in 14 children between 1955 and 1958, including five cases of fetal Minamata disease. At that time, the prevalence of cerebral palsy in Japan was 19 per 100,000 (0.02%), so the 14 cases found in the Minamata area showed how high the 7.5% per childbirth rate was [1–6].

In fact, this was not the first occurrence of patients with an unknown neurological disorder. Cases had appeared sporadically from around 1953 but were considered to be due to alcoholism or Japanese encephalitis in adult poisoning cases [3]. In 1954 a neighborhood cat suffered from excessive salivation, manifested general convulsions, and exhibited epilepsy-like behavior, jumping into the sea and drowning. It was said to have "cat dance disease." It was also reported that the cats in the area had disappeared and the number of rats increased rapidly [3, 5, 6].

A research group investigating the cause was launched, together with the Kumamoto University School of Medicine [7, 8]. In November 1956, poisoning by

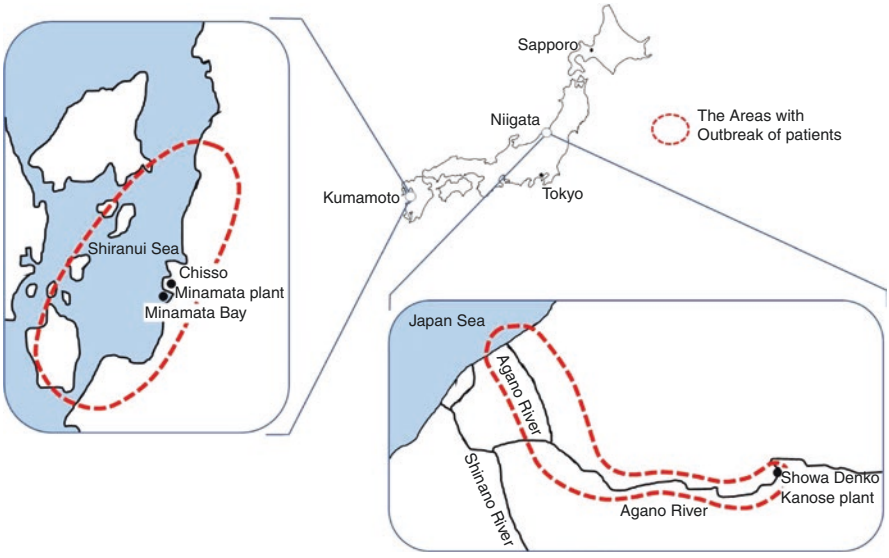


Fig. 1.1 The areas with outbreak of Minamata disease

heavy metals such as manganese, selenium, and cadmium was suspected, and experiments were conducted. It was a very difficult task, demanding much time and great efforts, because the researchers first had to establish methods for measuring these chemicals. Then they started toxicological experiments in animals. After that, based on the pathological diagnosis of a fatal case, it was reported by Prof. Tadao Takeuchi of Kumamoto University that it closely resembled an autopsied case of methylmercury poisoning (Hunter–Russell syndrome) [9, 10] in England reported by Drs. Donald Hunter and Dorothy S. Russell. In Minamata, damage to the central nervous system, particularly the cerebral cortex and cerebellar cortex, was notable. The disease was further characterized by notable damage to the calcarine region of the occipital lobe (visual center), the pre- and postcentral cortex (motor and sensory centers), and temporal cortex (auditory center), showing granular cell type cerebellar atrophy. In the peripheral nervous system, destruction and demyelination of dorsal roots and sensory neurofibers were characteristically notable. The organs of the dead patient contained a large amount of mercury [7, 8].

Time series data from the past to the present such as the transition of the acetaldehyde production volume at the Minamata plant and the number of patients occurring in parallel were also helpful for determining the cause. Unfortunately, no human biota samples were collected from the Minamata area during the exposure period of severe mercury pollution and methylmercury exposure levels could not be determined. Therefore, the time-course and regional distribution of pollution in Minamata were unknown. However, there is a traditional custom in Japan to keep the umbilical cord in a dried state as a birth commemoration. Dr. Masazumi Harada and Dr. Susumu Nishigaki obtained the umbilical cords of residents born in Minamata city to examine the relationship between methylmercury contamination from 1950 to 1959 and the onset of Minamata disease. They showed that the methylmercury concentration in stored umbilical cord tissue reflected the acetaldehyde production of the Chisso plant. As shown in Fig. 1.2 [11], the methylmercury concentration in stored umbilical cords exceeded 1 μg/g with increased production of acetaldehyde in the Chisso factory, and the high concentrations of methylmercury in stored umbilical cord tissues above 2 μg/g mostly coincided with the peak of the volume of acetaldehyde production in the plant.

In November 1959, as a result of elucidation of the cause by the Food Sanitation Investigation Committee of the Ministry of Health, the organic mercury theory was reported to the Minister of Health and Welfare. Nevertheless, measures were not taken to remediate the cause of Minamata disease. This was because the factory side presented new arguments such as “the poisoning theory due to rotten fish,” and investigation inside the factory was restricted. However, the company already knew the truth and hid it [3, 6, 12]. In the same year (1959), Dr. Hajime Hosokawa, the head of Chisso’s Minamata Hospital, fed cats with industrial waste from the factory for experimental elucidation of the pathogenesis and succeeded in producing Minamata disease with drainage from the acetaldehyde process. It was also confirmed by pathological findings. Waste water from other processes could not produce Minamata disease. However, release of the detailed data was stopped by the company, and his findings were not officially published in his lifetime. Nevertheless,

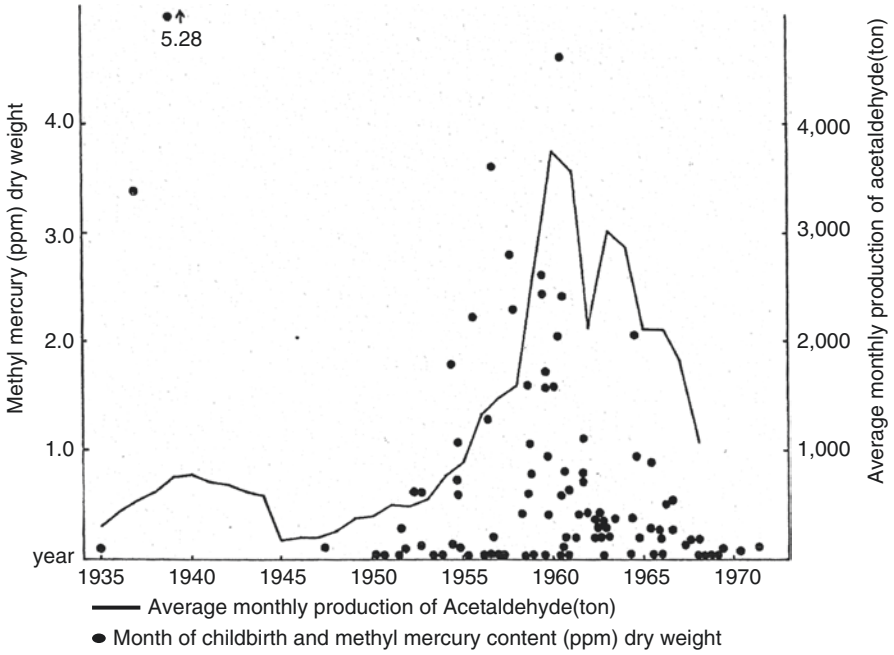


Fig. 1.2 Comparative chart of acetaldehyde production rate and methylmercury concentration in umbilical cords (From Nishigaki and Harada [11], with permission)

Hosokawa and his colleagues who did experiments had shown their experimental notes on, "the record of cat No. 400," to Dr. Ui Jun, a researcher in sanitary engineering and also a citizen activist, and his friend, Mr. Shisei Kuwabara, a famous photographer [12]. Thus, the intuition of Hosokawa, who had the sharp eyes of a physician scientist, linked ecological events to the human Minamata disaster. Many people are deeply impressed with Dr. Hosokawa's conscience as a scientist. In 2001, Dr. Komyo Eto, a pathologist who discovered autopsy specimens of the historic 1959 cat experiment by Hajime Hosokawa et al. reappraised their findings [13].

1.2 Outbreak of the Second Minamata Disease (Niigata-Minamata Disease)

In 1965, about 3,700 people were employed at the Chisso Minamata industrial plant, one of the biggest and most modern chemical plants in Japan. It was then producing chemical fertilizers, synthetic resins, plasticizers, and industrial chemicals. The plant used a variety of chemical substances in large quantities and discharged the waste water directly into a canal near Minamata Bay [2, 3, 5].

Ten years after the first official discovery of Minamata disease, in May 1965, the Ministry of Health in Niigata prefecture, which faces the Sea of Japan in the central

part of Honshu Island and is more than 1,000 km from Minamata in Kyushu (Fig. 1.1), were informed that "a central nervous system disease of unknown origin has occurred." This was strikingly similar to the cases of Minamata in Kumamoto prefecture. It is said to be the "second" Minamata disease due to methylmercury poisoning as a result to factory drainage by a major large company.

In September 1968, it was found that the source of the mercury pollution was the same acetaldehyde production process, used by the Showa Denko Plant Co., Ltd. located in the town of Kanase (now called Aka), about 60 km upstream from the mouth of the Agano River (Fig. 1.1). The mercury outflow mechanism was similar to that of the Chisso Minamata plant, but the residents of the basin area who ate river fish rather than sea fish became ill. In all, there were about 700 certified patients and more than 2,000 others patients. In Niigata, clinical neurological research from the beginning of the outbreak revealed that, in addition to severe cases with symptoms similar to those of Minamata disease, minor cases and atypical cases existed [14, 15]. A neurologist (Dr Kiyotaro Kondo) of Niigata University at that time reported that only one case of congenital Minamata disease was clinically suspected in Niigata, whereas Dr Masazumi Harada had found at least 64 cases in the Minamata area of Kumamoto prefecture by 1995 [16]. These numbers represent cases accepted by neurologists, not the numbers of those officially recognized for compensation.

In Minamata, the acetaldehyde process in which mercury was used as a catalyst at the Chisso Minamata plant was finally abandoned in May of 1968. However, if the experience of Minamata, where the disease appeared 12 years or more previously, had been made use of and serious corrective measures taken, would not the occurrence of the "second" Minamata disease itself have been prevented?

There was evidence of contamination of marine life close to the plant as early as 1925. Chisso Co., Ltd. had paid compensation to the fisherman's union but took no countermeasures whatsoever against contamination. At that time, acetaldehyde produced by the Chisso Minamata plant was an essential raw material for the growing Japanese chemical industry. As a result of company malfeasance, the central government acquiesced and the patients of Minamata and Niigata were sacrificed [3, 6, 12].

1.3 Pathological Differences Between Adults and Children

In fetal Minamata disease, severe neurodevelopmental symptoms and cerebral palsy-like symptoms are observed. Figure 1.3 shows differences in pathological findings (comparison of brain lesions) between adults and children. Histopathological lesions of the nervous system were similar to those in the adult type of Minamata disease. However, neuronal loss and hypoplasia of neurons, characterized by decreased size of neuron, were observed in parts of cerebral cortices [17]. Regarding the pathogenesis of Minamata disease, especially for the localized damages of the adult brain versus diffused damages in the fetus brain, Eto et al. also suggested that

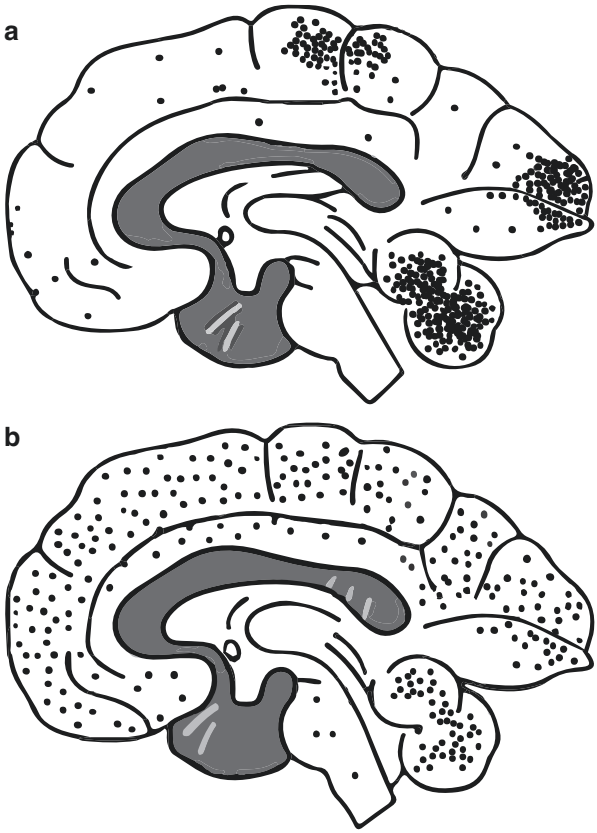


Fig. 1.3 Comparisons of the distributions of lesions among adult and fetal-type cases of Minamata disease (Modified from Takeuchi and Eto [8], with permission)

edema in the white matter near the deep sulci (calcarine, central, and sylvian fissures) may contribute to the selective damage, which usually were not shown with fetal or infantile cases [18].

Fetal Minamata disease is also called congenital Minamata disease. In severe child cases, the patient is bedridden with severe motor and intellectual disabilities. Cerebral palsy and severe abnormalities are usually not seen at birth, but they are noticed due to the delayed development of both mental and motor functions after birth. The mother's symptoms are generally mild. Why are there prenatal and childhood problems? The discovery of fetal Minamata disease, which causes serious damage to the fetus, but not the mother, and its special occurrence status have significant meaning in elucidating the development of fetal health problems caused by contamination with environmental chemicals and considering countermeasures.

Sakamoto et al. indicated why fetuses are at the high risk of methylmercury exposure. Methylmercury has a high affinity for the sulfhydryl (SH) group of the sulfur-containing amino acid cysteine and it becomes methylmercury having a similar structure to methionine, which is an essential amino acid and a cysteine conjugate. It is taken into the body via the neutral amino acid transport system and easily

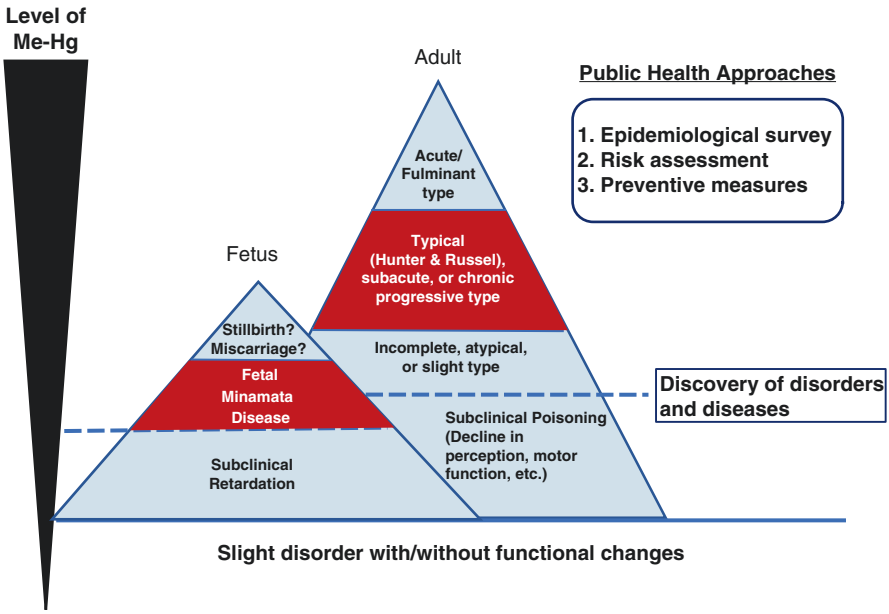


Fig. 1.4 Methylmercury exposure level and health impacts on humans in the heavily exposed community. The fetal level of MeHg is inferred from maternal exposed level. The important role of public health approaches includes the following: (1) Epidemiological survey focused on selected exposed populations, (2) Scientific risk assessments of chemicals, and (3) Preventive measures based on regulations

passes through the blood–brain barrier and the blood–placental barrier. In particular, since the fetus has a high demand for neutral amino acids, methylmercury is actively transported transplacentally to the fetus, and the mercury concentration in the hair of the fetus is 1.5–2 times higher than that of mother [19].

Figure 1.4 indicates what kinds of adverse health effects appear, as a result of exposure to methylmercury, comparing adults and children. The upper part of the iceberg presents the symptoms that the patient shows. Below that are subclinical symptoms due to relatively low-level exposure found in the residents of the area. This figure also shows the difference between public health and clinical medicine approaches to the entire exposed population. Although a reduced male proportion at birth was reported in two studies [20, 21] and there was an increase in stillbirths [22], no other epidemiological studies evaluated the impact of exposure on pregnancy outcomes in the Minamata area at the time of the outbreak. Increases of infertility and miscarriage cannot be ruled out with high concentration methylmercury exposure. However, community-based epidemiological studies on pregnancy outcomes with accurate exposure assessments of individuals were not possible in Japan because of the lack of environmental epidemiologists. Recently Yorifuji et al. observed a slight increase of spontaneous stillbirth and a decrease in the artificial stillbirth (abortion) rate in the city of Minamata followed by a reduced crude fertility

rate determined by descriptive retrospective analysis. The crude fertility rate in Minamata during the period 1955–1965 was significantly lower than that in the whole prefecture of Kumamoto. The impact on infant mortality was equivocal. These temporal trend analyses demonstrate a severe regional impact of methylmercury exposure on a series of birth outcomes in the Minamata area [23].

In a series on drug and chemical toxicology, Scharden reported a review on chemically induced birth defects [24]. From the early 1960s to the 1970s, many Japanese researchers, inspired by Minamata disease, found that methylmercury compounds induced a multiplicity of abnormalities, especially central nervous system types, in a variety of species. The eye is a target organ for this chemical. Central nervous system lesions mimicking the human condition in rats proved it to be a potent teratogen. In animal studies, a high incidence of resorptions and dead fetuses was noticed, whereas cleft palate was the most frequent malformation [25].

In contrast, adult cases, depending on the exposure level, are classified into five stages: (1) acute cases (fulminant type with turbidity, convulsions, etc.), (2) typical so-called adult cases in the case of organic mercury poisoning, Hunter–Russell syndrome with the three typical symptoms of visual field constriction, motor ataxia, and central sensory impairment, as reported by Hunter and Russell [9, 10], (3) a syndrome that has some of these symptoms, (4) a mild level of dysfunction, and (5) most minor chronic exposure cases, which, as a group, may have slightly decreased function compared to a control group. On the other hand, in the case of fetal exposure, even if the degree of the mother's disability is slight, the fetus has a weak blood–brain barrier and almost all nervous tissue is affected.

1.4 Impact of Fetal Minamata Disease: Why Are Fetuses and Children Vulnerable to Environmental Exposure?

According to the case reports of Minamata disease and Kanemi rice-bran oil disease (Yusho) described below, the fetal period is the most vulnerable to the environment and other environmental chemicals for many reasons [26, 27]: (1) Although there is a blood–placental barrier, many environmental chemicals move from the mother to the fetus. In fetuses, which do not have a detoxifying function and do not have excretory organs, chemical substances tend to accumulate, and toxicity is more pronounced. (2) Furthermore, in the brain in the early stage up to six months after birth, the blood–brain barrier is more fragile, and the effects of poisoning spread throughout the central nervous system. Development of brain neurons, such as synapse formation, is considered to be a developmental stage until childhood after birth. In addition, (3) during early childhood water intake per body weight is 7 times, food intake is 3–4 times, and lung ventilation is 2 times those of adults. That is why exposure intake through water, diet, and air is several times higher in children. Finally, (4) their height is short and exposure to toxicants such as house dust is high due to the behavioral characteristics of infants and children such as crawling on the floor [28].

Moreover, it is highly sensitive because of the time of window, when cells develop most. The fetal period, especially around 13 weeks after fertilization, is said to be the most susceptible organogenesis period, but it differs depending on the organ and function [24], the stages of human development are as follows: kidney, 4–40 weeks; central nervous system, 3 weeks to 20 years; lungs, 3–40 weeks with alveolar maturation taking 10 years reproductive system, 7–40 weeks with maturation in puberty. Thus, the development of the nervous system, respiratory function, and reproductive and immune systems is not only in the fetal stage but continues after birth until childhood or adolescence. Given these developmental stages of the child, the assessment of postnatal childhood exposure is also important.

1.5 Special Measures Act for Relief of Health Damage Related to Pollution

In 1969, the city of Minamata was designated as a region covered by the "Special Measures Act for Relief of Health Damage Related to Pollution." Over the years from 1974 to 1990, a project to remove about 150 sediments from Minamata Bay containing more than 25 ppm of total mercury and to construct a landfill site was carried out at a cost of ¥48.0 billion. There were 10,350 noncertified patients who were not legally recognized while having mercury poisoning symptoms, but who received compensation of ¥2,600,000 per person uniformly in 1994, as well as patients who had already died before detection. Thus, it is said that at least 20,000 people were affected by Minamata disease-related health disorders (11% of the total population of 180,000 in the Minamata area). Above all, patients with fetal Minamata disease were the worst affected, as neurons were lost during development. They will end their precious, but painful existences without ever having a human-like life [1, 3, 5].

In the company town, the patients were neglected and blamed by their community and people were divided over litigation. Of the certified patients in the Minamata area, at the end of 2016, more than 80% of the total of 2,282 patients who were recognized as suffering from Minamata disease had already died. Although not certified as having Minamata disease, more than 20,000 persons received aid from the government or were recognized as victims as a result of lawsuits. Even now, around 60 years after the first officially notified case, 2,139 others are still seeking legal compensation from the government [6].

1.6 What Should We Learn from the History of Minamata Disease?

A great deal of effort was spent to determine the clinical features in Minamata. However, in many cases, energy has been expended on the accumulation of only typical clinical cases of Hunter–Russell syndrome, and not on epidemiology

targeting the total polluted region, i.e., subclinical cases with functional disorders. There should have been a solid community epidemiological survey based on personal biological exposure assessment at the beginning of the outbreak. Efforts should also have been made to grasp the whole picture from the standpoint of public health. In addition, the cause of the escalating damage should have been determined. Elucidation based on social science was also necessary. When faced with such a situation, the following should be considered from the public health points of view as shown in Fig. 1.4.

1. Need for thorough environmental epidemiological survey in the early stage

In 1955, when the problem came to light, personal exposure assessments with biometric materials such as hair and blood and assessment of health effects based on the results, especially examination of exposure and biological effects, should have been done focusing on the local population, i.e., the entire exposed population. If they had been able to determine the prognosis according to the exposure concentration via follow-up, it could have obviated the need for long-term examination for medical recognition and damage compensation that continued for several decades after the outbreak, and confusion in court trials. We would have been able to solve many problems rapidly based on actual scientific follow-up data.

2. The important role of public health survey including long term follow-ups and risk assessments

We also need to understand the overall situation brought about by environmental pollution and look at the subclinical picture to recognize subclinical functional disorders. In the assessment of levels of exposure to environmentally harmful substances, it is necessary to not only look at the patient but also grasp the overall picture of the area, particularly inapparent poisoning in the first stage and disorders that occur with aging. In particular, a decrease in memory and motor function due to aging is remarkable and often observed in both central and peripheral nerves. More than 60 years after the outbreak, whether or not there is an increase in symptoms due to aging? That was also an important issue in recognition of Minamata disease.

One of the examples is our previous study to determine the residual effects of long-term exposure to mercury vapor. Neurobehavioral tests were given to ex-mercury miners about 18 years after the end of mercury exposures. Most subjects showed poorer performance with increasing age. For several neurobehavioral measures, ex-mercury miners with toxicosis showed more pronounced age-related trends toward deteriorating performance than did age–education matched community control subjects and ex-mercury miners without a history of mercury toxicosis. Longitudinal follow-up study on the interaction of aging and mercury toxicosis seems to be especially important as natural neuronal attrition may enhance the subclinical performance decrements related to previous mercury exposure [29].

3. Elucidating the cause of the great deal of damage extension.

What factors delayed the clarification of the truth? What factors significantly delayed measures to prevent the spread of damage? What factors have delayed the

resolution of practical issues such as victim security? To clarify them considering the socioeconomic, political, and cultural backgrounds is also necessary to prevent repeated occurrences of such disastrous and painful events. If elucidation of the problems related to companies and local communities, or government and administrative systems and the constitution are not sufficient, a new tragedy will be repeated in a different place, as in the case of the second Minamata disease in Niigata prefecture.

1.7 Yusho Outbreak (Kanemi Rice-Bran Oil Incident)

In 1968, in the Kitakyushu area of Japan, the Kanemi rice-bran oil incident occurred due to an accident in which PCB used for heating and deodorizing as a heat medium in the manufacturing process of edible oil leaked from a piping pinhole and mixed with the oil. However, the incident could not be explained only due to the PCB that was initially considered. Later, dioxins such as PCDD and PCDF were detected in a patient's blood, and dioxins produced by PCB heating were found to be the main cause [30–33].

PCBs and dioxins are classified as persistent organic pollutants (POPs) together with organochlorine pesticide and, for the purpose of reducing their amounts in the environment, the Stockholm Convention prohibits or restricts their production, use, import, and export. It took effect in 2014 and blood levels are gradually falling. However, POPs, once released into the environment, stay there for long periods such as several decades. Since the half-life in vivo is often several years or more, pollution has spread throughout the world from the Arctic to the Antarctic, across borders, continents, and oceans. Furthermore, in recent years, PFOS and PFOA, which are organic fluorochemicals frequently used in living environments as flame retardants, flame-proofing agents, and water repellents, have been recognized to be POPs, and their use has been restricted.

1.8 Authentic Birth Cohort Studies

If the exposure concentration of environmental chemicals is low, neither adults nor children think that they are ill, so it is rare for them to visit medical institutions by themselves. However, in the area of Minamata, it has been proved by a survey of school children that potential neurological deterioration has occurred in the population. Futatsuka et al. [34] compared the motor functions of junior high school students in the same grade in the Minamata disease area with a control area. They found that junior high school students in the contaminated area showed significant declines of hand dexterity, exercise agility, speech smoothness (articulatory function), as well as peripheral sensory dysfunction compared to students in a control area. It is important to clarify at what exposure level (from what concentration level)

such a decrease in neural function occurs and conversely at which concentration level it is safe or not in responding to the anxiety of the Japanese people whose main source of protein is fish and shellfish. In spite of that, for Minamata disease, epidemiological investigation was unfortunately not conducted sufficiently.

Risk assessments for exposure to low concentrations of mercury that were not identified in Japan's Minamata were conducted firstly in New Zealand, then on the 18 islands of the Faroe Islands in the North Atlantic. In New Zealand, prof. Tord Kjellstrom initiated a large scale epidemiological study, there the popular meal of fish-and-chips usually contain shark, but not regular fish. They found New Zealand children exposed to methylmercury in utero found 3-point decrement in IQ as well as alternation in behavior [35]. The mother's hair mercury concentration during pregnancy exceeded 6 ug/g, which level is about sixfold higher than the exposure limit used by the US EPA today [36].

There are few people in the global environmental health field who do not know the research done by professor Philippe Grandjean et al., and their cohort is known as a representative birth cohort. The birth cohort study that has been conducted in the Faroe Islands, where people capture and eat whales, started in 1986 and consists of five cohorts in all. A total of 2300 mothers and children participated in the first stage. The mercury exposure level of this island population (mother group) was estimated to be about one-fifth to one-sixth that of Minamata disease patients, but none showed symptoms similar to Minamata disease.

A survey of the neurodevelopment of 7-year-old children was conducted in 1993–1994 [37, 38]. The same survey of the children was repeated when they were 14 years old in 2000 and 2001 [39]. The mercury concentration in cord blood of children was strongly associated with language, attention, and memory-related variables at age seven. There were also tests that showed a strong relationship with the mercury index at the age of seven. Even at the age of 14 years, the reaction time was found to be significantly related to the cord blood mercury concentration. The results obtained from the Faroe studies have scientifically indicated that marine-derived methylmercury causes mild impairment in childhood development [40].

1.9 "Our Stolen Future" Widens the Perspective

There is global concern about children's environment and health and the environmental effects of chemical exposure. The beginning was the book "Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival?" by Theo Colborn, Dianne Dumanoski, and John Peterson Myers published in 1996 [41]. Typically, Colborn et al. warned about the endocrine disrupting properties of polychlorinated chemicals (PCBs) and dioxins. The fetus in utero was the most sensitive to these chemical exposures. Consequently, the potential risks of birth defects, developmental delay, disruption of thyroid and reproductive hormones, and alterations of immunological and allergic functions were suggested to be caused by exposure to these chemicals; indeed, these effects "steal" children's future.

It has been suggested that PCBs (polychlorinated biphenyls) and dioxins inhibit hormone homeostasis to the hypothalamus-pituitary-gonadal axis by acting on sex steroid hormone receptors and cause endocrine disruption. In the past, however, the effects on sex hormones were reported to be due to high exposure from industrial areas and poisoning accidents. These reports [42–44] suggested that fetal exposure to PCBs and dioxins might have antiandrogenic effects. However, these were studies of areas that were exposed to high concentrations and the effects of exposure were not evaluated and the fetal exposure concentration was not measured. Analysis of the adolescent sex hormones of children born to mothers suffering from rice oil disease (Yusho) in Japan, caused by contamination of the oil by PCBs and their heat-degraded by-products, polychlorinated dibenzofurans [PCDFs], and ter- and quaterphenyls [PCTs and PCQs]) among Yucheong patients in Taiwan revealed that boys had decreased free testosterone to estradiol (T/E2) ratios and girls had increased estradiol levels. However, at that time we did not know there was a possibility that even background low-level exposure to POPs could cause these endocrine-disrupting effects in children.

1.10 Full-Scale Birth Cohort Studies Launched in Japan from 2001 and 2002

1.10.1 The First Prospective Cohorts in Japan

Both the Tohoku Study of Child Development (TSCD) and the Hokkaido Study of Environment and Children's Health: Malformation Development and Allergy are ongoing cohort studies that began in 2001 in the northern part of Japan. The TSCD is mainly focused on exposure of mothers and their offspring (n = 1,300) to POPs, especially to methylmercury and PCBs [45]. They found important developmental landmarks had significant negative correlations with PCB and methylmercury exposure during gestation. Even low methylmercury exposure levels induced subtle developmental effects in children [46–48]. On the other hand, the Hokkaido study consists of two prospective birth cohorts, the Sapporo cohort (n = 514) and the Hokkaido large-scale cohort (n = 20,940). The most remarkable feature of this study is that it is a prospective cohort study of the background levels in areas, Sapporo city and Hokkaido prefecture that are not polluted in Japan.

The primary goals of the Hokkaido study [49] are to first examine the potential negative effects of perinatal environmental chemical exposures on birth outcomes, including congenital malformations and growth retardation; second, to evaluate the development of allergies, infectious diseases, and neurodevelopmental disorders and perform longitudinal observations of the children's physical development to clarify the causal relationships between these outcomes and environmental chemicals; third, to identify individuals genetically susceptible to environmental chemicals; and finally, to identify the additive effects of various environmental factors in our daily life such as secondhand smoke exposure and low folate intake during early

pregnancy. Recently, we reported our progress in the Hokkaido study with cohort profiles updated in 2013 [50] and 2017 [51]. For the last 18 years we have followed pregnant women and their offspring, measuring various environmental chemicals; i.e., PCB, OH-PCB and dioxins, perfluorinated compounds (PFASs), organochlorine pesticides, phthalates, bisphenol A, and mercury.

In the Sapporo cohort, the levels of 29 dioxin and dioxin-like PCB congeners, 58 other PCB congeners, and 5 hydroxylated PCB congeners in maternal blood and breast milk were measured. We discovered that the toxic equivalency (TEQ) concentrations of dioxin and other specific congeners of PCDF or PCDD have effects on birth weight [52], infant neurodevelopment [53–55], and immune function [56, 57]. There were significant gender differences in these effects. These results on dioxin congeners suggest that male infants are more susceptible to those chemical exposures than female infants. However, some other chemicals such as PFOS had stronger effects in females; e.g., a birth weight decrease was found only in female infants [58]. Interestingly, it was found maternal genetic polymorphisms, for instance, in *AHR*, *CYP1A1*, and *GSTs*, that significantly modified the dioxin concentrations in maternal blood, suggesting different dioxin accumulation in the bodies of individuals with these genotypes, which would lead to different dioxin exposure levels. These genetic susceptibility factors influenced the body size of children born to mothers who either smoked or were passively exposed to tobacco smoke [59].

Regarding newborn anthropometric measurement data, such as birth weight and birth size, there was a lack of associations between prenatal exposure to PCBs and methylmercury and birth weight. Although no association was observed with birth weight, the risk of small for gestational age (SGA) by weight decreased with increased concentrations [60]. The incidence of SGA babies by weight was 4.9%. The median concentrations of total PCBs and hair mercury were 108 ng/g lipid and 1.41 μg/g, respectively. We observed that the risk of SGA by weight decreased with increasing mercury concentrations in regression analyses with adjustment for polyunsaturated fatty acids [60, 61]. These results suggest that the beneficial effect of consuming seafood, especially fish, on essential nutrition may mask the adverse effects of methylmercury on birth size. The concentrations of PCBs had no association with birth size.

1.10.2 Endocrine Disruptions Caused in Utero

Even low level dioxin or PFOS and PFOA exposure had significant effects on thyroid hormone [62, 63]. Steroid and reproductive hormone levels were investigated in the Sapporo cohort [64–69]. The relationship between prenatal exposure to dioxin, PFOS, PFOA, organochlorine pesticide, DEHP, and bisphenol A and the levels of reproductive and steroid hormones in cord blood were investigated. An increase in maternal dioxin-like compounds (DLCs) was related to decreased T/E2 ratios and SHBG and inhibin B levels, and increased AA/GC ratios and FSH and

DHEA levels in male cord blood. Prenatal exposure to DLCs alters steroidogenesis and suppresses inhibin B in male cord blood [65]. However, relationships between maternal DLCs and cord blood hormones differ between boys and girls. Prenatal exposure to PFOS, but not PFOA, showed an inverse dose–response relationship with cortisol and cortisone concentrations. PFOS and PFOA showed both positive and inverse associations with the levels of several reproductive and steroid hormones [66, 67]. Similarly, MEHP was associated reduced levels of several reproductive and steroid hormones [68], especially in boys. Bisphenol A was associated with reduced prolactin levels [69]. Further studies are required to clarify whether the effects of in utero exposure to DLCs and other chemical compounds on these hormones extend into infancy and puberty.

1.11 The Twenty-First Century is the Era of Birth Cohort Studies

The twenty-first century is the era of birth cohort studies around the globe. Historically, many birth cohorts were established in Europe. As described before, Children's Health and the Environment in the Faroes started their initial recruitment in 1986. In 1990, the Avon Longitudinal Study of Parents and Children (ALSPAC) (http://www.bristol.ac.uk/alspac/) recruited their children and recently, children of ALSPAC are called to participate in the study as the next generation. From the late 1990s to the early 2000s, national cohorts in Denmark (Danish National Birth Cohort: DNBC) (https://www.dnbc.dk/) and Norway (The Norwegian Mother and Child Cohort Study: MoBa) (https://www.fhi.no/en/studies/moba/), in addition to cohorts in France (French Longitudinal Study of Children: ELFE) (https://www.elfe-france.fr/en/), the Netherlands (Generation R (https://generationr.nl/researchers/) and the Amsterdam Born Children and their Development [ABCD] Study (https://academic.oup.com/ije/article/40/5/1176/656509)), and Spain (Spanish Environment and Childhood Research Network [INMA] Study (https://www.sciencedirect.com/science/article/pii/S143846390700020X)), etc. were started. In the USA, the Michigan cohort started in 1980 (https://precisionhealth.umich.edu/workgroups/cohort-development/), and many others such as CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas (https://cerch.berkeley.edu/research-programs/chamacos-study)), and the New York City Mothers and Newborns Study Cohort started in the late 1990s (https://ccceh.org/our-research/featured-nyc-research-findings). Independently, each cohort provides voluminous evidence in this field. Moreover, comparison between cohorts, data combination, and meta-analyses provide even more rigid evidence.

In Asia, several birth cohorts were launched in early 2000, and the Birth Cohort Consortium of Asia (BiCCA) (http://www.bicca.org/) was co-established in 2011 by the principal investigators of three birth cohorts in Asia: the Taiwan Birth Panel Study (TBPS, Taiwan) (https://tbps/tw.org/), the Mothers and Children's Environment study (MOCEH, Korea) (https://www.niehs.nih.gov/research/programs/heh/partnerships/

network/cohorts/moche/index.cfm), and the Hokkaido Study on Environment and Children's Health (Hokkaido Study, Japan) (https://www.cehs.hokudai.jp/en/project/pro04). As of April 2019, 31 cohorts in 16 countries have joined the BiCCA [70, 71]. In addition, a national cohort of 100,000 mother-child pairs has begun in Japan (http://www.env.go.jp/chemi/ceh/en/) and Korea started recruiting in 2011, and the birth data have already been fixed. Ko-CHENS (Korean CHildren's Environmental Health Study) also started recruiting in 2015, and domestic and international cohort collaboration has also begun to progress.

1.12 Environment and DOHaD Paradigm

The Developmental Origins of Health and Disease (DOHaD) approach originally focused on nutritional insults during the developmental stage and followed adult diseases such as coronary heart disease. However, it has become a multidisciplinary field that examines how environmental factors acting during the phase of developmental plasticity interact with genotypic variations; for instance, the correlations among epigenetics, the effects of intrauterine exposure to environmental chemicals and developmental factors related to health later in life, dysfunction, and disease [72]. New findings resulting from the integration of "environment" into the DOHaD paradigm are promising, especially for the prevention of disease/dysfunction focusing on both clinical and public health implications.

Acknowledgements I would like to express my gratitude to Mr. Kim M. Barrymore, who helped with revisions for this chapter. This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18gk0110032.

References

1. Smith WE, Smith MA. Minamata: a warning to the world. London: Chatto & Windus Ltd.; 1975. p. 180–7.
2. Harada M. Congenital Minamata disease: intrauterine methylmercury poisoning. Teratology. 1978;18:285–8.
3. Harada M. Minamata byo. Tokyo: Iwanami-Shoten; 1972.
4. Matsumoto H, Koya G, Takeuchi T. Fetal Minamata disease. A neuropathological study of 2 cases of intrauterine intoxication by a methyl mercury compound. J Neuropathol Exp Neurol. 1965;24(4):563–74.
5. Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol. 1995;25(1):1–24.
6. Kishi R. History, cause and measures of Minamata disease. In: Kishi Editorial Supervision, editor. New preventive medicine and public health. 4th ed. Tokyo: Nankodo; 2018. p. 14–9.
7. Minamata Disease Research Group. Minamata disease. Kumamoto: Medical School of Kumamoto University; 1968.

8. Takeuchi T, Eto K. The pathology of Minamata disease. Fukuoka: Kyushu University Press; 1999.
9. Hunter D, Bomford R, Russell DS. Poisoning by mercury compounds. Q J Med. 1940;9:193–213.
10. Hunter D, Russell DS. Focal cerebellar and cerebellar atrophy in a human subject due to organic mercury compounds. J Neurol Neurosurg Psychiatry. 1954;17(4):235–41.
11. Nishigaki S, Harada M. Methylmercury and selenium in umbilical cords of inhabitants of the Minamata area. Nature. 1975;258(5533):324–5.
12. Kuwabara S. The Minamata disaster. Tokyo: Fujiwara-Shoten; 2013.
13. Eto K, Yasutake A, Nakano A, Akagi H, Tokunaga H, Kojima T. Reappraisal of the historic 1959 cat experiment in Minamata by the Chisso Factory. Tohoku J Exp Med. 2001; 194:197–203.
14. Tsubaki T. In: Irukayama K, editor. Minamata disease: methylmercury poisoning in Minamata and Niigata, Japan. Tokyo: Kodansha; 1977.
15. Tsubaki T, Takahashi H, editors. Recent advances in Minamata disease studies: methylmercury poisoning in Minamata and Niigata, Japan. Tokyo: Kodansha; 1986.
16. Kondo K. Congenital Minamata disease: warning from Japan's experience. J Child Neurol. 2000;15:458–64.
17. Eto K, Oyanagi S, Itai Y, Tokunaga H, Takizawa Y, Suda I. A fetal type of Minamata disease –an autopsy case report with special reference to the nervous system. Mol Chem Neuropathol. 1992;16(1-2):171–86.
18. Eto K, Yasutake A, Kuwana T, Korogi Y, Akima M, Shimozeki T, Tokunaga H, Kneko Y. Methylmercury poisoning in common marmosets –a study of selective venerability within the cerebral cortex. Toxicol Pathol. 2001;29(5):565–73.
19. Sakamoto M, Tatsuta N, Izumo K, Phan PT, Vu LD, Yamamoto M, et al. Health impacts and biomarkers of prenatal exposure to methylmercury: lessons from Minamata, Japan. Toxics. 2018;6:45.
20. Sakamoto M, Nakano A, Akagi H. Declining Minamata male birth ratio associated with increased male fetal death due to heavy methylmercury pollution. Environ Res. 2001;87(2):92–8.
21. Doi R, Kobayashi T, Ohno H, Harada H. Sex ratio of congenital Minamata disease patients (in Japanese). Jpn J Hyg. 1985;40:306.
22. Itai Y, Fujino T, Ueno K, Motomatsu Y. An epidemiological study of the incidence abnormal pregnancy in area heavily contaminated with methylmercury. Environ Sci. 2004;11:83–97.
23. Yorifuji T, Kahisma S, Suryadhi A, Abudureyimu K. Temporal trends of infant and birth outcomes in Minamata after severe methylmercury exposure. Environ Pollut. 2017; 231:1586–92.
24. Dicarlo JF, Oechme WF, Schaeden LJ. Chemically induced birth defects. Drug Chem Toxicol. 1985;2:622–32.
25. Dwivedi RS, Ianaccone PM. Effects of environmental chemicals on early development. In: Korach KS, editor. Reproductive and developmental toxicology. New York: Marcel Dekker, Inc.; 1998.
26. Landorigan PJ, Etzel RA. Children's environmental health-a new branch of pediatrics on text book of children's environmental health. Oxford: Oxford University Press; 2014. p. 3–17.
27. Etzel RA, Landorigan PJ. Children's exquisite vulnerability to environmental exposures. In: Textbook of children's environmental health. Oxford: Oxford University Press; 2014. p. 18–27.
28. Ait Bamai Y, Araki A, Kawai T, Tsuboi T, Yoshioka E, Kanazawa A, Cong S, Kishi R. Comparisons of urinary phthalate metabolites and daily phthalate intakes among Japanese families. Int J Hyg Environ Health. 2015;218:461–70.
29. Kishi R, et al. Residual neurobehavioural effects associated with chronic exposure to mercury vapour. Occup Environ Med. 1994;51:35–41.
30. Kuratsune M, Yoshimura T, Matsuzaka J, Yamaguchi A. Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ Health Perspect. 1972;1:119–28.

31. Masuda Y. Approach to risk assessment of chlorinated dioxins from Yusho PCB poisoning. Chemosphere. 1996;32(3):583–94.
32. Yoshimura T. Yusho in Japan. Ind Health. 2003;41(3):139–48.
33. Nagayama J, Todaka T, Hirakawa H, Hori T, Kajiwara J, Yoshimura T, Furue M. Polychlorinated dibenzofurans as a causal agent of fetal Yusho. Chemosphere. 2010;80(5):513–8.
34. Futatsuka M, Matsushita T, Arimatsu Y, Ueda A, Misumi J, Tomio T, Nomura S. Physical function of junior high school students in organic mercury contaminated area. Jpn J Publ Health. 1973;20(6):299–314.
35. Grandjean P. Only one chance; how environmental pollution impairs brain development--- and how to protect the brains of the next generation. Oxford: Oxford University Press; 2013.
36. Kjellström T, Kennedy P, Wallis S, et al. Physical and mental development of children with prenatal and mental development of children with prenatal exposure to mercury from fish, stage 2, interviews and psychological tests at age 6. Stockholm: National Swedish Environment Protection Board; 1989.
37. Grandjean P, Weihe P, White RF, Debes F, Araki S, Murata K, Sørensen N, Dahl D, Yokoyama K, Jørgensen PJ. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol. 1997;19(6):417–28.
38. Grandjean P, Weihe P, Burse VW, Needham LL, Storr-Hansen E, Heinzow B, Debes F, Murata K, Simonsen H, Ellefsen P, Budtz-Jørgensen E, Keiding N, White RF. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Public Health Rep. 1999;114(6):512–5.
39. Debes F, Budtz-Jørgensen E, Weihe P, White RF, Grandjean P. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. Neurotoxicol Teratol. 2006;28(3):363–75.
40. Choi AL, Mogensen UB, Bjerve KS, Debes F, Weihe P, Grandjean P, Budtz-Jørgensen E. Negative confounding by essential fatty acids in methylmercury neurotoxicity associations. Environ Sci Technol. 2011;45(3):1121–6.
41. Colborn T, Dumanoski D, Myers JP. Our stolen future: are we threatening our fertility, intelligence, and survival? New York: Dutton; 1996.
42. Hsu PC, Lai TJ, Guo NW, Lambert GH, Guo YL. Serum hormones in boys prenatally exposed to polychlorinated biphenyls and dibenzofurans. J Toxicol Environ Health A. 2005;68(17-18):1447–56.
43. Cao Y, Winneke G, Wilhelm M, Wittsiepe J, Lemm F, Fürst P, Ranft U, Imöhl M, Kraft M, Oesch-Bartlomowicz B, Krämer U. Environmental exposure to dioxins and polychlorinated biphenyls reduce levels of gonadal hormones in newborns: results from the Duisburg cohort study. Int J Hyg Environ Health. 2008;211(1-2):30–9.
44. Xu P, Lou X, Ding G, Shen H, Wu L, Chen Z, Han J, Han G, Wang X. Association of PCB, PBDE and PCDD/F body burdens with hormone levels for children in an e-waste dismantling area of Zhejiang Province, China. Sci Total Environ. 2014;499:55–61.
45. Nakai K, Suzuki K, Oka T, Murata K, Sakamoto M, Okamura K, Hosokawa T, Sakai T, Nakamura T, Saito Y, Kurokawa N, Kameo S, Satoh H. The Tohoku Study of Child Development: a cohort study of effects of perinatal exposures to methylmercury and environmentally persistent organic pollutants on neurobehavioral development in Japanese children. Tohoku J Exp Med. 2004;202(3):227–37.
46. Suzuki K, Nakai K, Sugawara T, Nakamura T, Ohba T, Shimada M, Hosokawa T, Okamura K, Sakai T, Kurokawa N, Murata K, Satoh C, Satoh H. Neurobehavioral effects of prenatal exposure to methylmercury and PCBs, and seafood intake: neonatal behavioral assessment scale results of Tohoku study of child development. Environ Res. 2010;110(7):699–704. https://doi.org/10.1016/j.envres.2010.07.001.
47. Tatsuta N, Murata K, Iwai-Shimada M, Yaginuma-Sakurai K, Satoh H, Nakai K. Psychomotor ability in children prenatally exposed to methylmercury: the 18-month follow-up of Tohoku study of child development. Tohoku J Exp Med. 2017;242(1):1–8. https://doi.org/10.1620/tjem.242.1.
48. Tatsuta N, Nakai K, Murata K, Suzuki K, Iwai-Shimada M, Kurokawa N, Hosokawa T, Satoh H. Impacts of prenatal exposures to polychlorinated biphenyls, methylmercury, and lead on

intellectual ability of 42-month-old children in Japan. Environ Res. 2014;133:321–6. https://doi.org/10.1016/j.envres.2014.05.024.
49. Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, Kurahashi N, Tamaki J, Endo T, Sengoku K, Nonomura K, Minakami H, Health for the Hokkaido Study on Environment and Children's. Cohort profile: the Hokkaido Study on environment and children's health in Japan. Int J Epidemiol. 2011;40(3):611–8.
50. Kishi R, Kobayashi S, Ikeno T, Araki A, Miyashita C, Itoh S, Sasaki S, Okada E, Kobayashi S, Kashino I, Itoh K, Nakajima S. Members of the Hokkaido Study on Environment and Children's. Ten years of progress in the Hokkaido birth cohort study on environment and children's health: cohort profile - updated 2013. Environ Health Prev Med. 2013;18(6):429–50.
51. Kishi R, Araki A, Minatoya M, Hanaoka T, Miyashita C, Itoh S, Kobayashi S, Ait Bamai Y, Yamazaki K, Miura R, Tamura N, Ito K, Goudarzi H. The Hokkaido birth cohort study on environment and children's health: cohort profile – updated 2017. Environ Health Prev Med. 2017;22(1):46.
52. Konishi K, Sasaki S, Kato S, Ban S, Washino N, Kajiwara J, Todaka T, Hirakawa H, Hori T, Yasutake D, Kishi R. Prenatal exposure to PCDDs/PCDFs and dioxin-like PCBs in relation to birth weight. Environ Res. 2009;109(7):906–13.
53. Nakajima S, Saijo Y, Kato S, Sasaki S, Uno A, Kanagami N, Hirakawa H, Hori T, Tobiishi K, Todaka T, Nakamura Y, Yanagiya S, Sengoku Y, Iida T, Sata F, Kishi R. Effects of prenatal exposure to polychlorinated biphenyls and dioxins on mental and motor development in Japanese children at 6 months of age. Environ Health Perspect. 2006;114(5):773–8.
54. Nakajima S, Saijo Y, Miyashita C, Ikeno T, Sasaki S, Kajiwara J, Kishi R. Sex-specific differences in effect of prenatal exposure to dioxin-like compounds on neurodevelopment in Japanese children: Sapporo cohort study. Environ Res. 2017;159:222–31.
55. Minatoya M, Nakajima S, Sasaki S, Araki A, Miyashita C, Ikeno T, Nakajima T, Goto Y, Kishi R. Effects of prenatal phthalate exposure on thyroid hormone levels, mental and psychomotor development of infants: The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2016;565:1037–43.
56. Miyashita C, Sasaki S, Saijo Y, Washino N, Okada E, Kobayashi S, Konishi K, Kajiwara J, Todaka T, Kishi R. Effects of prenatal exposure to dioxin-like compounds on allergies and infections during infancy. Environ Res. 2011;111(4):551–8.
57. Miyashita C, Ait Bamai Y, Araki A, Itoh S, Minatoya M, Kobayashi S, Kajiwara J, Hori T, Kishi R. 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. 2017;610-611:191–9.
58. Washino N, Saijo Y, Sasaki S, Kato S, Ban S, Konishi K, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect. 2009;117(4):660–7.
59. Kobayashi S, Sata F, Miyashita C, Sasaki S, Ban S, Araki A, Goudarzi H, Kajiwara J, Todaka T, Kishi R. Dioxin-metabolizing genes in relation to effects of prenatal dioxin levels and reduced birth size: the Hokkaido Study. Reprod Toxicol. 2017;67:111–6.
60. Miyashita C, Sasaki S, Ikeno T, Araki A, Ito S, Kajiwara J, Todaka T, Hachiya N, Yasutake A, Murata K, Nakajima T, Kishi R. Effects of in utero exposure to polychlorinated biphenyls, methylmercury, and polyunsaturated fatty acids on birth size. Sci Total Environ. 2015;533:256–65.
61. Miyashita C, Sasaki S, Saijo Y, Okada E, Kobayashi S, Baba T, Kajiwara J, Todaka T, Iwasaki Y, Nakazawa H, Hachiya N, Yasutake A, Murata K, Kishi R. Demographic, behavioral, dietary, and socioeconomic characteristics related to persistent organic pollutants and mercury levels in pregnant women in Japan. Chemosphere. 2015;133:13–21.
62. Baba T, Ito S, Yuasa M, Yoshioka E, Miyashita C, Araki A, Sasaki S, Kobayashi S, Kajiwara J, Hori T, Kato S, Kishi R. Association of prenatal exposure to PCDD/Fs and PCBs with maternal and infant thyroid hormones: The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2018;615:1239–46.
63. Kato S, Itoh S, Yuasa M, Baba T, Miyashita C, Sasaki S, Nakajima S, Uno A, Nakazawa H, Iwasaki Y, Okada E, Kishi R. Association of perfluorinated chemical exposure in utero with

maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environ Health Prev Med. 2016;21(5):334–44.
64. Araki A, Miyashita C, Mitsui T, Goudarzi H, Mizutani F, Chisaki Y, Itoh S, Sasaki S, Cho K, Moriya K, Shinohara N, Nonomura K, Kishi R. Prenatal organochlorine pesticide exposure and the disruption of steroids and reproductive hormones in cord blood: The Hokkaido Study. Environ Int. 2018;110:1–13.
65. Miyashita C, Araki A, Mitsui T, Itoh S, Goudarzi H, Sasaki S, Kajiwara J, Hori H, Cho K, Moriya K, Shinohara N, Nonomura K, Kishi R. Sex-related differences in the associations between maternal dioxin-like compounds and reproductive and steroid hormones in cord blood: The Hokkaido Study. Environ Int. 2013;117:175–85.
66. Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, Nakazawa H, Nonomura K, Kishi R. The association of prenatal exposure to perfluorinated chemicals with glucocorticoid and androgenic hormones in cord blood samples: The Hokkaido Study. Environ Health Perspect. 2017;125(1):111–8.
67. Itoh S, Baba T, Yuasa M, Miyashita C, Kobayashi S, Araki A, Sasaki S, Kajiwara J, Hori T, Todaka T, Fujikura K, Nakajima S, Kato S, Kishi R. Association of maternal serum concentration of hydroxylated polychlorinated biphenyls with maternal and neonatal thyroid hormones: The Hokkaido Birth Cohort Study. Environ Res. 2018;67:583–90.
68. Araki A, Mitsui T, Goudarzi H, Nakajima T, Miyashita C, Itoh S, Sasaki S, Cho K, Moriya K, Shinohara N, Nonomura K, Kishi R. Prenatal di(2-ethylhexyl) phthalate exposure and disruption of adrenal androgens and glucocorticoids levels in cord blood: The Hokkaido Study. Sci Total Environ. 2017;581-582:297–304.
69. Minatoya M, Sasaki S, Araki A, Miyashita C, Itoh S, Yamamoto J, Matsumura T, Mitsui T, Moriya K, Cho K, Morioka K, Minakam H, Shinohara N, Kishi R. Cord blood bisphenol A levels and reproductive and thyroid hormone levels of neonates: The Hokkaido Study on Environment and Children's Health. Epidemiology. 2017;28(Suppl 1):S3–9.
70. Kishi R, Zhang JJ, Ha EH, Chen PC, Tian Y, Xia Y, Tsuchiya KJ, Nakai K, Kim S, Hong SJ, Hong YC, Lee JR, Mohamed HJBJ, Parajuli RP, Adair LS, Chong YS, Guo YL, Wang SL, Nishijo M, Kido T, Tai PT, Nandasena S. Birth cohort consortium of Asia (BiCCA). Current and future perspectives. Epidemiology. 2017;28(1):S19–34.
71. Kishi R, Araki A, Minatoya M, Itoh S, Goudarzi H, Miyashita C. Birth cohorts in Asia: the importance, advantages, and disadvantages of different-sized cohorts. Sci Total Environ. 2018;615:1143–54.
72. Gluckman P, Hanson M, editors. The developmental origins of health and disease. Cambridge: Cambridge University Press; 2006.

Part II
Adverse Health Effects on Human Developing Organs Caused by Environmental Chemicals-the Role That Chemicals Might Play

Chapter 2
Environmental Exposures and Adverse Pregnancy-Related Outcomes

Machiko Minatoya, Tomoyuki Hanaoka, and Reiko Kishi

Abstract It has been known that pregnant women are exposed to a number of environmental chemicals and studies have reported that these environmental chemicals are detected from pregnant women of various population. Environmental exposures such as air pollution, pesticides, solvents, heavy metals, and chemicals including persistent organic pollutants (POPs) have implicated in adverse pregnancy-related outcomes. In addition to these environmental chemicals, phthalates and bisphenol A (BPA) are known as ubiquitous environmental chemicals and their endocrine disrupting effects have been reported from animal studies. These chemicals can impact the ability to become pregnant and sustain a healthy pregnancy. Taking trend of environmental chemical levels over the years into consideration is of cardinal importance for investigating adverse birth outcomes because oftentimes associations can be found at high exposure levels such as accidental or occupational settings. From individual studies and meta-analysis, systematic reviews of recent years, evidences on outdoor air pollution during pregnancy, and adverse birth outcomes have been strengthened. Especially, fairly good evidence for maternal exposure to $PM_{2.5}$ pollution during pregnancy is associated with increased risk of adverse birth outcomes. Heavy metal exposures and PFOA exposure during pregnancy may moderately be associated with adverse birth outcomes; however, not sufficient investigation has been conducted for substitutions for PFOS and PFOA. Besides, it is important to consider concentrations of PFAS in association with adverse birth outcomes as PFOS concentration is decreasing since the Stockholm convention. Inconsistent findings have been reported for phthalates and BPA exposure during pregnancy and birth outcomes. Some of the studies mentioned

M. Minatoya (✉)
Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Hokkaido, Japan

Faculty of Health Sciences, Hokkaido University, Sapporo, Hokkaido, Japan
e-mail: mminatoya@cehs.hokudai.ac.jp

T. Hanaoka · R. Kishi
Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Hokkaido, Japan

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_2

different influence between male and female infants or sex-specific of environmental exposure on birth outcomes. Studies on genomic analysis, transcriptome analysis, proteome analysis, and epigenome analysis should be accelerated to elucidate mechanisms of observed results from epidemiological studies.

Keywords Miscarriage/stillbirth · Preterm birth · Low birth weight · Small for gestational age · Environmental chemicals · Outdoor air pollution · Heavy metals · Organochlorine compounds · Per- and polyfluoroalkyl substances (PFAS) · Polybrominated diphenyl esters (PBDEs) · Organophosphate pesticides and pyrethroid · Phthalates · Bisphenol A

2.1 Adverse Pregnancy-Related Outcomes Overview

The fetus has been recognized as particularly vulnerable to the effects of environmental factors that disrupt developmental processes. Exposure to environmental contaminants can permanently change the body structure, physiology, and metabolism. Miscarriage, stillbirth, preterm birth (PTB), low birth weight (LBW), and small for gestational age (SGA) continue to be major public health problems, despite improvement in health care. These adverse pregnancy-related outcomes have been attracted attention due to Developmental Origins of Health and Disease (DOHaD) theory, which hypothesizes that gestational and perinatal environment is associated with long-term health consequences [1]. Studies have reported that adverse pregnancy-related outcomes including PTB, LBW, SGA were risk factors for diseases in later life. Environmental exposures such as air pollution, pesticides, solvents, heavy metals, and chemicals including persistent organic pollutants (POPs), per- and polyfluoroalkyl substances (PFAS), phthalates, bisphenol A (BPA) have implicated in adverse pregnancy-related outcomes. These chemicals can impact the ability to become pregnant and sustain a healthy pregnancy. It has been known that pregnant women are exposed to a number of environmental chemicals and studies have reported that these environmental chemicals are detected from pregnant women of various population [2]. Environmental exposures and adverse pregnancy-related outcomes have been described in many review articles [3, 4]. Up to now, however, good epidemiological evidence for developmental effect of prenatal exposures was available for only a few pollutants. In addition, there are a large number of reviews on specific environmental exposures during pregnancy and its outcomes. These reviews provide evidence between specific environmental exposures and adverse pregnancy outcomes, but normally limited for other environmental exposures. In this chapter, 4 of the adverse pregnancy outcomes: miscarriage/stillbirth, PTB, LBW, and SGA, are discussed in association with environmental risk factors. Health consequences of these adverse birth outcomes also are described.

Furthermore, there has been suggested that specific genotypes may modify the effects of chemical exposure on health outcomes [5, 6] and gene–environmental interaction will be featured.

2.2 Definitions of Adverse Pregnancy-Related Outcomes

2.2.1 Miscarriage/Stillbirth

Sporadic miscarriage, the loss of an embryo or fetus before 20 weeks of pregnancy, is the most common complication of pregnancy, affecting approximately 15% of all clinically recognized pregnancies [7]. Risk factors for pregnancy loss include but are not limited to advanced maternal age, hormonal imbalances, immunological interactions, and uterine anatomic abnormalities [8]. Certainly, lifestyle factors such as tobacco smoke and alcohol usage are generally well accepted as contributing causes of miscarriage [8]. The definition of stillbirth recommended by World Health Organization (WHO) for international comparison is a baby born with no signs of life at or after 28 weeks gestation. An estimated 2.6 million stillbirths occurred annually, of which 98% occur in low- and middle-income countries and 75% in sub-Saharan Africa and south Asia [9]. The number of stillbirths has declined by 19.4% between 2000 and 2015. Half of all stillbirths occur during labor and birth. Most result from preventable conditions including maternal infections, non-communicable diseases, and obstetric complications. The etiology of stillbirth is likely to be multifactorial and may come from both intrinsic and extrinsic characteristics [10].

2.2.2 Preterm Birth (PTB)

Babies born before the 37th completed week of pregnancy are defined as PTBs and babies born before the 32nd completed week of pregnancy are considered very preterm. PTBs are rising globally; as estimated 15 million babies are born preterm every year, representing a PTB rate of 11.1% [11]. Despite a reduction in the number of live births, the estimated number of PTBs in 65 countries in Europe, the Americas, and Australia increased from 2.0 million in 1990 to nearly 2.2 million in 2010. However, estimation is complicated by differences in measurement of gestational age, preterm definitions, as well as differences in data collection and reporting. Preterm deliveries are heterogeneous in terms of assumed proximal causes or associated maternal conditions (ischemic placental diseases, infectious or inflammatory context, etc.) and of clinical context (premature rupture of the chorioamniotic membranes, preterm labor, induced labor, or cesarean section for medical indications, e.g., to try to protect the mother or the fetus) [12].

2.2.3 Low Birth Weight (LBW)

Birth weight is the weight of the newborn measured immediately after birth. As defined by WHO, birth weight of less than 2500 g (5.5 lb) is considered LBW. Birth weight less than 1500 g (3.3 lb) is considered very LBW (VLBW). This is based on epidemiological studies regarding the increased risk of death to the infant and serves as a benchmark for international comparisons. On average across Organization for Economic Co-operation and Development (OECD) countries about 6.5% of live births are recorded as LBW [13]. The prevalence of LBW infants has increased in most OECD countries since 1990. The reasons for this increase include, increase in the number of multiple births, partly as a result of the rise in fertility treatments, the increased age of mothers at childbirth, and an increase in smoking among young women from the 1970s onwards.

2.2.4 Small for Gestational Age (SGA)

SGA fetuses or newborns are those smaller in size than normal for their gestational age most commonly defined as a weight below the 10th percentile for the gestational age. This classification was originally developed by a 1995 WHO expert committee, and the definition is based on a birth weight-for-gestational-age measure compared to a gender-specific reference population [14, 15]. The incidence of infants who are SGA worldwide is 9.7% and the percentage is increasing [16]. However, multiple criteria also have been used such as less than the 10th and 5th percentile in weight, length, or head circumference. Another definition of SGA is parameters more than 2 standard deviations (SDs) below the mean, or 2.3rd percentile [17, 18]. In 2007, a consensus meeting that included representatives from seven international pediatric endocrinology societies, as well as a representative from obstetrics, perinatology and neonatology, pediatrics, epidemiology, and pharmacology recommended that SGA be defined as more than 2 SDs below the mean for weight and/or length [18]. It has been more than ten years since consensus was published; the definition has not been widely applied or accepted. According to an article [19], researchers have continuously used various parameters to define SGA and findings from different definition of SGA have been published in the international journals. More research is needed to better understand the pathophysiology of SGA and also to investigate potential early pregnancy biomarkers, which might have utility in early pregnancy prediction of SGA.

2.3 Risk Factors

The fetus requires substrates for growth and production of energy. Gases and nutrients pass freely to the fetus from the mother through the placental membrane. Glucose is a primary source of energy for fetal metabolism and growth; amino acids

are also required. Insulin is required for the metabolism of glucose and is secreted by the fetal pancreas. Insulin, human growth hormone, and some small polypeptides (e.g., insulin-like growth factor I) are believed to stimulate fetal growth. In general, factors operating throughout pregnancy tend to produce intrauterine growth restriction (IUGR) and small neonates, whereas factors operating during the last trimester usually produce underweight neonates with normal length and head size. Ubiquitous exposure to environmental chemicals may contribute to fetal growth restriction.

2.3.1 Outdoor Air Pollution

A number of studies regarding air pollution and stillbirth have been conducted. Studies focused on pollutants including $PM_{2.5}$, carbon monoxide (CO) and sulfur dioxide (SO_2), nitrogen dioxide (NO_2), black carbon, polycyclic aromatic hydrocarbons (PAHs). Overall, consistent finding of negative association between stillbirth and air pollution has been reported [20–28]. In addition, quite a number of studies have investigated association between air pollution and PTB [29–34]. Maternal exposure to ambient fine particulate matter is believed to increase the risk of PTB by increasing systemic oxidative stress and inflammation, impairing placentation, causing endocrine disruption (e.g., disturbing the pituitary–adrenocortical–placental system), and increasing maternal susceptibility to infections [35]. Meta-analyses and systematic reviews have revealed that air pollution including $PM_{2.5}$, PM_{10}, SO_2 is associated with an increased risk of PTB [36–38]. Studies have shown that higher PAH levels were associated with an increased risk of PTB [39, 40]. Meta-analyses and systematic reviews showed that maternal exposure to fine particulate air pollution increased the risk of term LBW [37, 41–45]; however, results for O_3 and SO_2 were found to be less consistent [37, 46]. Associations between specific components of $PM_{2.5}$ and LBW have been reported [25]. Toxic heavy metal exposures including Pb, Cd, mercury (Hg), and As from air pollutants during pregnancy have been examined in association with LBW; however, findings are inconsistent [47–56]. There are a number of studies regarding SGA and fetal growth reduction in association with maternal exposure to air pollution [37, 57]. Recently, high PAHs exposure from e-waste area was associated with reduced birth size [58]. Studies of PAH exposure and adverse birth outcomes indicate a relationship; however, additional studies that address the importance of PAH compared to other components of the complex mixture should be considered. Despite the fact that several studies have found significant evidence linking air pollution and adverse birth outcomes, only little is known about the mechanism of action. Air pollutants can cross the placental barrier to reach the fetus directly and consequentially weaken relevant transplacental functions. It has been suggested that air pollutants can cause an inflammatory response, oxidative stress [59], an allergic immune response, and a reduction in heart rate variability [60].

2.3.2 Heavy Metals

Low and high level exposure to lead (Pb) and arsenic (As) exposure during pregnancy was associated with stillbirths [61, 62]. A number of epidemiological studies from Bangladesh, India, Sweden, Hungary, Mongolia, and the USA have reported an increased risk of stillbirth in association with chronic As exposure [50]. A review included 12 literatures on As exposure of pregnant women and risk of stillbirth found that 9 out of 12 supported a positive association [50]. For exposure to Hg and adverse birth outcome, studies of severe methylmercury poisoning occurred in Minamata area during the 1950s and 1960s have been conducted. In Minamata area, increased spontaneous and decreased artificial stillbirth rates were observed [63, 64]. In addition, reduced male proportion at birth was observed [63, 65]. Pb has been associated with a range of adverse health outcomes, and, notably, there seems to be no threshold of exposure for most effects [66]. Pb exposure in relation to birth outcomes has been well-reviewed in the article of early 1990s. The review reported that Pb exposure was likely associated with increased risk of PTB, and that the effects were dose-dependent [67]. Increasing Pb levels were significantly associated with PTB in the studies of relatively higher exposure levels; however, studies of Pb exposure in lower ranges and PTB are less consistent. No significant association was found in both cross-sectional and prospective studies [47, 68]. It was pointed out that blood Pb levels during certain time points during pregnancy may be especially predictive of PTB. In a study, a 1 SD increase in blood Pb levels measured during the second trimester was associated with significantly increased odds of PTB (OR = 1.75, 95%CI = 1.02, 3.02). However, associations with first or third trimesters and cord blood were not statistically significant [69]. Contrary, one study observed that elevated maternal first trimester blood Pb levels were significantly associated with increased odds of PTB (OR = 1.41, 95%CI = 1.08, 1.84) [70]. Addressing critical windows of exposure is a particularly important aspect of understanding chemical exposure associations with PTB; however, the evidence is still inconclusive. Results from studies examining the relationship between Cd exposure and PTB have been conflicting. An ecologic study found no significant elevation in odds of shortened gestation in women who lived in areas with elevated exposure to Cd [71]. Similarly, more recent study of a small cohort in China found no significant associations between Cd measured in maternal whole blood, cord blood, or placenta and risk of PTB [72]. In a study in Bangladesh, women residing in areas with high exposure to As had significantly higher PTB rates compared to women residing in low exposure areas [73]. A study in Taiwan found an elevated odd of PTB in association with residence in an area with a history of high well-water levels of As [74]. Relatively large ecologic study (n = 9890) did not find a significant change in odds of PTB (OR = 1.02, 95%CI = 0.72, 1.44) with residence in a village with high exposure in China [75]. Studies of metal and metalloid exposure and PTB provide evidence for an effect of Pb at higher levels, but studies at levels more consistent with current environmental exposure levels, or for Cd, As, or Hg, are inconclusive. Hg exposure has been linked to PTB. The Pregnancy Outcomes and Community Health Study observed a significantly elevated odds of very PTB in association with Hg hair levels ≥90th percentile (OR = 3.0, 95% CI = 1.3, 6.7), although the OR for delivery

<37 weeks gestation was not significant [76]. A study conducted in China reported that LBW occurred more frequently in infants with higher Cd levels in cord blood than in those exposed to lower levels of Cd in cord blood [77]. Similarly, a study including healthy non-smoking pregnant women found that birth weight was inversely correlated with Cd levels in both maternal blood and cord blood [78]. Three studies with large sample sizes from the USA have all found that maternal blood Pb levels were associated with the risk of decreased birth weight [47, 79, 80]. A considerable number of epidemiologic studies have examined the association between prenatal exposure to Hg and birth size. Some found no association [81–84], while the others reported negative association between Hg exposure and birth weight [85–87]. Studies with relatively higher levels of exposure tended to observe adverse outcomes. High maternal blood Cd levels during pregnancy were associated with increased ORs of infants being born SGA and these observed effects were independent of cotinine-defined smoking status [88]. However, the other study reported that Cd levels were not significantly associated with SGA [89]. As exposure was also reported to be associated with SGA [56, 90]. A large cohort study in Norway including 62,941 pregnant women reported that those in the highest Hg exposure group showed increased risk of SGA [87]. Contrary, a population-based study found no association between maternal blood Hg levels and SGA among 15,444 pregnant women [91] and concluded that in a Japanese population, which has a relatively higher blood Hg level than reported in Western population, reduced birth size was not associated with Hg levels. Another birth cohort study from Japan that investigated the risk of SGA with Hg concentration suggested that beneficial effect of essential nutrition may mask the adverse effects of Hg on birth size [92]. A study reported a result as women with high blood Pb were at 4.2 times increased risk for delivering SGA newborn (95% CI = 1.3–13.9) [93]. Overall, effects of exposure to toxic heavy metals on SGA are not clear and more studies are necessary.

Each heavy metal has different biological functions and several possible mechanisms linked to adverse birth outcomes have been suggested. Exposure to toxic metals may affect the fertilized egg or zygote implantation into the lining of the uterus or disrupt early pregnancy placentation. Toxic heavy metals may accumulate in placental transfer cells causing a decrease in uterine blood flow, and decrease the transfer of nutrients to the fetus, and thus affect fetal growth [94]. Future studies should also be designed to identify whether there are increased risks of adverse birth outcomes associated with exposure to heavy metals at relatively low levels common among the general population as most studies have focused on relatively high exposure levels.

2.3.3 Organochlorine Compounds

1,1,1-trichloro–2,2′bis(*p*-chlorophenyl) ethane (DDT) is one of the most well-known EDCs and is widespread in the environment; in fact, some countries are still using DDT in vector control [95, 96]. Spontaneous abortion has been correlated with DDT exposure in multiple studies. DDT may increase a risk of miscarriage or stillbirth via suggested role in decline in sperm counts, increase in time to conception, and even intrauterine growth restriction [95, 97, 98]. Most human studies on miscarriage and DDT have

correlated DDT or DDT metabolites with pregnancy outcomes. Some studies show limited impact of DDT and dichlorodiphenyl dichloroethane (DDE) on miscarriage [95, 99, 100]. One of the largest studies of DDT and DDE in relation to miscarriage found an adjusted OR of miscarriage per 60 μg/L increase in DDE of 1.4 (95% CI, 1.1–1.6) [101]. A case–control study found an OR for miscarriage of 1.13 (95% CI, 1.02–1.26) in association with maternal DDE levels [99]. A prospective study found a relative odds of pregnancy loss of 1.17 (95% CI = 1.05–1.29) associated with a 10 ng/g increase in serum total DDT [100]. Overall, these studies consistently show an increase in spontaneous abortion in association with higher serum levels of DDT or DDT metabolites. Dioxin-related toxins including polychlorinated biphenyls (PCBs) still persist in the environment, despite their use being banned. These compounds can act not only as estrogen agonists or antagonists but also can have androgenic properties and these effects are considered to have the potential to cause miscarriage. Animal studies have constantly shown fetal loss. Similar findings have been reported in epidemiological studies. In a study of Yusho incident, a statistically significant increase in miscarriage was noted, with OR ranging from 1.6 (95% CI = 1.1–2.33) to 2.52 (95% CI = 0.92–6.87) depending on the type of PCB measured [102]. Similarly, the Yu-sheng accident and Vietnamese exposure to Agent Orange have shown an increase in spontaneous abortion [103, 104]. Contrary, no increase in miscarriage was observed after an explosion at a chemical factory in Seveso, Italy that involved dioxin [105]. Studies of exposed Vietnamese women exposure to Agent Orange have shown an increase in miscarriage and premature deliveries [106]. An increase in miscarriage and other adverse reproductive outcomes in association with PCBs exposure are largely reported in retrospective studies. Further longitudinal studies are needed to better understand the reproductive repercussions of PCB on pregnancy outcomes. A study showed exposure to a mixture of PCBs and PCDFs affected stillbirth [107]. Studies reported maternal use of pesticide as a risk factor of stillbirth [108, 109] and organochlorine pesticides (OCPs) has been detected from brain samples of stillbirth infants [110]. There may be sex-specific effects of exposure to pesticides on birth outcomes; however, the evidence is very limited. The strongest evidence for a relationship between DDT exposure and PTB comes from a cohort study of the period when DDT use was at its peak in the USA and it found that increased maternal exposure to DDT was significantly associated with increased OR of preterm delivery, and that there was a dose-dependent effect [111]. However, more recent studies that investigated relationships between DDT exposure and PTB showed comparatively null results. The latest study, however, found significantly elevated DDE in preterm vs. term [112]. Several studies also examined the association between PTB and hexachlorobenzene (HCB). A study conducted in a population with high levels of HCB through air pollution observed a significant difference in exposure levels between cases and controls [113]. A study in an agricultural population with elevated exposure levels also found an inverse association between HCB exposure and gestational age [114]. However, others did not find the association [112, 115]. Another organochlorine pesticide, hexachlorocyclohexane (HCH) has been investigated as well. HCH exposure was found at higher levels in cases of PTB compared to term births in several studies [116, 117]. While relatively small case–control studies reported association between PCB exposure and PTB [113, 118], others did not find associations [119, 120]. In a large

meta-analysis examining the relationships between POP exposure and several birth outcomes, no association was observed between PCB-153 and gestational age in any individual study or overall [121]. Despite high levels of exposure in Seveso, Italy where extremely large amount of 2,3,7,8-TCDD was accidentally released, the elevated odds of PTB observed in association with TCDD serum measures were not considered significant [105]. Contrary, two studies of accidental exposure to dioxin, with limited exposure and outcome measurements, have suggested an association between dioxins and PTB [106, 122]. A number of studies examining birth weight in relation to dioxins and organochlorines compounds exist; however, most of these studies only reported some associations between exposure to these chemicals and birth weight and findings were inconsistent among studies [48, 121]. Several studies reported a negative association between DDT levels and birth weight [111, 123, 124]. One study which assessed 29 congeners levels of PCDDs/PCDFs and DL-PCBs reported a significant adverse effect between total PCDDs toxic equivalents (TEQ) levels and total PCDFs TEQ levels [125]. In addition, only among male infants, significant adverse associations with birth weight were found for total PCDDs TEQ level, total PCDDs/PCDFs TEQ level, and total TEQ level [125]. The same group reported that polymorphisms in AHR and CYP1A1 (rs4646903) were associated with maternal dioxin concentrations [5, 126] and further assessed gene–environmental interaction and observed adverse effects of maternal GSTM1 null genotype on birth weight in the presence of dioxins exposure during pregnancy [5, 127]. On the other hand, several studies did not find increased levels of DDT and DDE to be significantly associated with infant birth weight [113, 128–130]. Findings on exposure to HCH and LBW have been also inconclusive [94]. While occupationally and accidentally poisoned women have showed an increased risk of LBW, studies of prenatal low-level exposure to PCBs and LBW have produced inconsistent results [92, 119, 123, 128, 131–133]. Cord blood DNA methylation level changes in H19 and LINE-1 in association with prenatal exposure to specific non-dioxin-like PCBs with sex-specific manner have been suggested [5, 134]. This may indicate the importance of investigating epigenetic changes in association with environmental chemical exposures and birth weight. HCB exposure modestly increased ORs SGA in girls [135]; contrary, PCB-153 and HCB were associated with higher ORs for SGA and stronger associations among male infant [136]. One study provided evidence that genetic variation and its interaction with organochlorine pesticide exposure may increase the risk of SGA [137].

2.3.4 Per- and Polyfluoroalkyl Substances (PFAS)

A number of recent studies examined the relationship between PFOA and PFOS exposure and PTB. A study conducted where a relatively high proportion of PTB observed no significant differences in median concentrations of PFOS or PFOA in term compared to preterm samples [138]. Similarly, no significant change in odds of PTB in association with PFOA exposure was reported from the study where drinking water was sourced from a contaminated facility [139]. A recent study in the Danish National Birth Cohort estimated a nearly twofold increase in risks of PTB for the higher quartiles

of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) exposure [140]. In addition, risk of PTB was increased for perfluorononanoic acid (PFNA), perfluoroheptane sulfonate (PFHpS), and perfluorodecanoic acid (PFDA) in higher exposure ranges [140]. The opposite was suggested from other study; PFOS and PFOA were each associated with decreased ORs of PTB [141]. Other study in Taiwan reported an increased OR of PTB in association with PFOS levels while no significant associations were observed for PFOA, PFNA, or PFUA [142]. A study of e-waste contaminated area in China observed significantly higher levels of PFOA in maternal serum from preterm compared to term deliveries [143]. Reduced birth weight in association with exposure to PFOA during pregnancy seemed to be consistent with findings across the studies including a systematic review [144, 145]; however, magnitude of reduction in birth weight varied among studies and there have been no strong evidence for the risk of LBW. There has been indicated that the effects of prenatal PFOA exposure on birth size could be mediated through DNA methylation [146]. Findings on exposure to PFOS and birth weight are also inconsistent [145]. For example, one of the earliest reported presented that PFOS levels (median = 5.2 ng/mL) were negatively correlated with birth weight [147] and exposure to PFOS may disrupt adipokine, particularly adiponectin, which is related to fetal growth [148]. However, since the Stockholm convention, PFOS levels are gradually decreasing [149, 150] and the same group found no significant correlation between PFOS levels and reduced birth weight. These studies indicated the importance of taking trend of PFOS concentrations into account for investigating the association between exposures and birth outcomes. In addition, production of PFOS and PFOA has been regulated, instead, substituted compounds have been used and detected from pregnant women; however, there are almost no information on these emerging PFAS in association with birth weight. Further studies to elucidate the association between these emerging PFAS and reduced birth weight are warranted along with examining pathways. One study suggested that some PFAS were associated with elevated risks for LBW, but these estimates were less precise [140]. Prenatal exposure to PFOA was associated with SGA and the findings are moderately consistent [135, 136]. PFOS levels were associated with increased ORs of SGA in newborns of mothers who smoked during pregnancy, while an inverse association was found in those of non-smoking mothers [135]. These previous studies indicate the importance of investigating gene-environmental interaction and other contributing factors when conducting association study. The mechanisms underlying these associations have not been established, several potential pathways have been proposed linking prenatal PFAS exposure to impaired fetal development and birth size in humans including hormone disruption, altered lipid metabolism, immunotoxicity in pregnant women, and direct fetal toxicity [94].

2.3.5 Polybrominated Diphenyl Esters (PBDEs)

Little is studied on polybrominated diphenyl ethers (PBDEs), which are widely used as flame retardants in consumer products. PBDEs were reported to be associated with stillbirth [151]. One study conducted e-waste recycling area in China observed

significantly higher levels of various individual and summed PBDE congeners in cord blood from adverse birth outcome pregnancies including stillbirth, PTB, and LBW compared to normal pregnancies. This study provides suggestive evidence for future investigation of the association between PBDE exposure and PTB [151]. Several studies including cross-sectional studies have reported LBW in association with PBDEs exposure during pregnancy; however, findings are inconsistent regarding PBDE congeners [152, 153]. There are too little studies to provide the evidence.

2.3.6 Organophosphate Pesticides and Pyrethroid

Organophosphate pesticides are one class of these compounds, including chlorpyrifos, diazinon, malathion, parathion, and others, that are currently in common use in agricultural settings. A study conducted in aguricultural area with heavy use of prsticides observed neither individual nor summed organophosphates or metabolites were associated with a change in odds of PTB, decreased cholinesterase (ChE) levels in umbilical and maternal blood were found to be associated with increased risk of preterm birth [154]. No significant associations were identified between exposure to organophosphates as well as other pesticides and birth outcomes in a cohort of 2246 births at agricultural area [155]. A recent study reported that first or second trimester exposure to individual pesticides (e.g., glyphosates, paraquat, imidacloprid) or exposure to 2 or more pesticides in the three chemical classes were associated with a small increase in risk for PTB and the associations were stronger for female offspring [156]. Occupational exposure to pesticides found association with LBW or reduced birth weight in some studies [155, 157–160]. Contrary, some studies showed no association between pesticides such as pyrethroids exposure and birth weight [161–163]. Only a small number of studies on pyrethroid pesticide exposure and birth outcomes were found and findings from these studies are inconclusive. Moreover, one study reported a positive association for birth weight in association with maternal urinary concentration of 3-phenoxybenzoic acid (3-PBA), suggesting an intrauterine growth promoting effect [164]. These findings may suggest the possibility of relations between high level exposure to certain pesticide and LBW; however, evidence for causal relationship between low level exposure to pesticide during pregnancy and LBW is still inadequate.

2.3.7 Phthalates

Phthalates have been associated with developmental abnormalities of the male reproductive system, miscarriage, endometriosis, and low sperm counts [8]. Interaction with PPAR and decrease in aromatase activity have been theorized to contribute to adverse reproductive outcomes [165, 166]. In epidemiological study, elevated urinary concentration of mono-ethylhexyl phthalate around the time of conception was associated with an increase in miscarriage, with an OR of 2.87 (95% CI, 1.09, 7.57) in the

highest quartile of exposure [167]. An increase in miscarriage rates was found in the first and fourth quartile of eight different urinary phthalate metabolites levels [168]. In addition to adverse pregnancy outcomes, abnormal reproductive development in association with phthalate exposure during pregnancy has been reported in animal studies. Many of these effects are thought to be secondary to aromatase inhibition or other hormonal mimicry. Further epidemiological studies with larger size are warranted. Studies that investigated various phthalate metabolites and different exposure period continuously provided different findings on the risk of PTB. Some showed increased risks [169–173], while, a prospective study found that elevated urinary levels of DEHP metabolites were associated with decreased risk of preterm delivery [174]. In addition, phthalates may interact with other known risk factors for spontaneous or placental PTB such as maternal smoking, maternal race, and fetal sex, and the potential interactions between phthalates and these factors should be studied more closely. A study of relatively larger number of participants from three birth cohort studies identified that one of the phthalate metabolites (mono(2-ethyl-5-hydroxyhexyl) phthalate; MEHHP) was associated with reduced birth weight [175]. This suggests that different types of phthalate may have different influence on birth weight. Further investigation focuses on individual phthalate exposure as well as phthalates as a group exposure should be carried out. Meantime, further investigation on pathways should be investigated. Recently, disruption of adipokines such as leptin and adiponectin has been suggested from cohort studies [176–178] and which also have been found in animal studies [179]. This maybe a key in between prenatal exposure to phthalates and changes in birth weight. A recent study found that greater MiBP and MEOHP were associated with an overall higher SGA risk, greater MEHP was associated with higher SGA risk in whites but not in African Americans, and the associations for MiBP and ΣDBP varied by infant sex [180]. Exposure assessment using biomarkers is limited because phthalates are rapidly metabolized in the human body. Using a single urine sample may lead to measurement error and bias in results. Utilization of multiple exposure measurements throughout pregnancy would improve these estimates and also help to identify potentially sensitive time points in gestation.

2.3.8 Bisphenol A

BPA has been linked to reproductive disorders. Alteration of expression of genes associated with implantation has been associated with BPA as BPA is able to weakly bind the estrogen receptor [181, 182]. As a result of its estrogen agonist properties, an increase in expression of estrogen-regulated genes such as HOXA10, B3integrin, and ITGB3, which are known to be important for normal implantation, is found with BPA. BPA has also been shown to impact endocrine signaling by downregulating expression of ERα and PR. Endometrial angiogenesis is also impacted by BPA by downregulating vascular endothelial growth factor expression [181, 182]. These studies suggest that BPA has the potential to disrupt implantation, with the potential downstream effects of miscarriage and antenatal complications. Epidemiological studies on BPA exposure and miscarriage are very limited. The relative risk of loss

for sporadic miscarriage was 1.97 (95% CI, 1.08–3.59) and for recurrent loss was 3.33 (95% CI, 1.04–10.71) in the highest quartile of serum BPA concentration [183]. It has been reported that a higher urinary BPA level is associated with recurrent miscarriage; however, samples were not collected during pregnancy for the miscarriage group [184]. These data suggest a critical need for larger epidemiological studies to confirm an association between BPA exposure and miscarriage. Studies for BPA exposure and a risk of PTB have provided inconsistencies. A positive association between urinary BPA concentration of pregnant women and PTB and association was sex-specific [185–188]. Contrary, some studies reported no association. Given these situations, evidence for BPA exposure during pregnancy as risk factor for PTB is considered insufficient. BPA is also known as EDC and have been investigated in terms of exposure during pregnancy and birth weight. Occupational cohort study reported that infants born from BPA exposed mothers had lower birth weight and the relation was dose–response manner [189]. Some studies found association only among specific sex [190, 191], some studies found increased birth weight [192] or U-shaped association [193] and some studies found no association [194, 195]. The evidence of BPA affecting birth weight is equivocal. One study observed an elevated risk of SGA along with adverse action of leptin and adiponectin in male neonates in association with maternal BPA exposure [190]. Other cohort studies also observed disruption of adipokines including adiponectin and leptin in sex-specific manner [176, 178], thus, further studies focus on these metabolic biomarkers in association with BPA exposure and birth outcomes may be of importance.

2.4 Health Issues of Adverse Birth Outcomes

PTB is associated with strongly increased perinatal mortality and long-term morbidity [196]. PTB is the reason for LBW and is an important indirect cause of neonatal deaths. Preterm infants are at increased risk of developing later cardiovascular and metabolic disease independent of whether or not they have experienced IUGR [197, 198]. Furthermore, children who are born prematurely have reduced insulin sensitivity, a risk factor for type 2 diabetes millets (T2DM), irrespective of whether their weight was appropriate for gestational age (AGA) or SGA [199]. A review found that PTB is one of the robust risk factors for T2DM [200]. In addition to its significant contribution to mortality, many survivors of PTB face a lifetime of disability, including neurodevelopmental/behavioral effects, learning disabilities, and visual and hearing problems as well as higher risks of non-communicable diseases [201]. Preterm infants almost universally experience growth restriction after birth and are on average 1–2 standard deviations below the mean weight for corrected age by their time of hospital discharge [59]. Despite this growth restriction, preterm infants have greater adiposity at term-equivalent age compared to term birth infants [202, 203]. Altered fat accumulation in preterm infants, adiposity may represent a more accurate marker for the subsequent development of adverse health outcomes than birth weight alone [204]. PTB is one of the most consistent clinical surrogates

for low nephron numbers and is associated with an increased risk of hypertension, proteinuria, and kidney disease later in life [205]. This relationship is amplified by the development of acute kidney injury in preterm infants, which may further reduce nephron numbers soon after birth, as well as by rapid catch-up growth or overfeeding during infancy or childhood in children born small, which may further augment the risk of hypertension and chronic kidney disease and predispose to obesity and T2DM later in life [205]. For child behavioral problems and psychiatric disorders, a recent study identified that among children born extremely PTB, those with severe fetal growth restriction appear to be at increased risk of multiple cognitive and behavioral dysfunctions at age 10 years [206]. The other study observed that very PTB (VPTB) children at 13 years of age show an atypical neuronal activation during spatial working memory (WM), specifically related to manipulation of spatial information in WM [207]. Moreover, bronchopulmonary dysplasia is associated with delayed structural brain maturation in preterm infants [208]. Contrary, a systematic review concluded that there is a lack of evidence concerning risk factors for behavior problems and psychiatric disorders among VPTB population and identified the need for further research examining the etiology of disorders of psychological development in PTB population as influence of perinatal risk factors on cognitive development of VPTB appears to diminish over time as environmental factors become more important [209, 210]. Childhood neurobehavioral development can be more influenced by postnatal environmental factors rather than being PTB, and it is difficult to distinguish prenatal factors from postnatal factors. A recent review mentioned that most of the studies included showed increased risk for asthma and decreased allergic rhinitis in those born late PTB. LBW contributes to 60–80% of all neonatal deaths. However, despite the increase in the number of LBW infants, medical care for newborns has been particularly successful in reducing infant mortality. LBW infants are more likely to have underdeveloped lungs and breathing problems, heart problems, immature and improperly functioning livers, too many or too few red blood cells, inadequate body fat, leading to trouble maintaining a normal body temperature, feeding problems, and increased risk of infection [211]. A recent systematic review of 39 publications supports rapid postnatal catch-up growth of LBW neonates as a more important factor than LBW alone in the genesis of cardiovascular disease risk later in life [212]. The inverse association between LBW and later cardiovascular and metabolic disease risk could be explained by the DOHaD hypothesis: inadequate nutrition in utero could “program” the fetus with permanent changes in structure, physiology, and metabolism to increase short-term survival. However, when exposed to the ex utero environment, where the nutrient supply may be more abundant, these adaptations are mismatched, conferring an increased risk of later disease [204]. Because many of the effects of LBW are due to being born immature and unprepared for life outside the womb, morbidities associated with LBW often overlap with those of PTB. Epidemiological studies indicate that there is a correlation between LBW and hypertension in adulthood. The mechanism that links LBW and hypertension is multifactorial including delayed nephrogenesis, genetic factors, sympathetic hyperactivity, endothelial dysfunction, elastin deficiencies, insulin resistance, and activation of renin–angiotension system [213]. An umbrella review presented one

convincing evidence on an increased risk for all-cause mortality, highly suggestive evidence on an increased risk for wheezing in childhood and coronary disease, suggestive evidence on asthma in adulthood, RSV-related acute lower respiratory infections in childhood, and chronic kidney disease in association with LBW [214]. Relatively weak evidence on diastolic blood pressure, testicular cancer, T2DM, metabolic syndrome, pneumonia in childhood, school-age asthma, systolic blood pressure was also presented [214]. A systematic review and meta-analysis provided strong consistent evidence that LBW is associated with lower forced vital capacity in adult [215]. Babies that are born SGA have often experienced IUGR [216]. In short term, SGA is associated with increased mortality, lung disease, hypertension, necrotizing enterocolitis, poor thermoregulation, hypoglycemia, and polycythemia [17, 18]. In long term, SGA is risk for insulin resistance, T2DM, cardiovascular disease, chronic kidney disease, neurodevelopmental and cognitive impairments, developmental delays, behavioral problems, and adult short stature [17]. In children born SGA, poor nutritional conditions during gestation can modify metabolic systems to adapt to expectations of chronic undernutrition. These children are potentially poorly equipped to cope with energy-dense diets and are possibly programmed to store as much energy as possible, leading to later obesity, metabolic syndrome, disturbed regulation of normal puberty, and early onset of cardiovascular disease [217]. Babies born SGA as a result of maternal or placental nutrient restriction have consistently shown an altered pattern of fat accumulation [216]. Observation that infants born to poorly nourished mothers are often small but with greater deficits in skeletal muscle mass than in fat mass may suggested that fat accumulation is not affected as much as lean body mass [218]. Other studies have reported that newborn infants that are SGA have a reduced absolute fat mass when compared to AGA infants, reflecting reduced in utero fat accumulation [219]. Infants who are born SGA normally show faster postnatal growth, known as a concept of catch-up growth as a result of recovery from undernutrition in utero. Children who were born SGA but achieved normal weight and height by 2 years of age showed greater accumulation of total body and abdominal fat between 2 and 4 years of age compared to AGA children [220]. However, early postnatal growth is also influenced by genetic factors as well as nutrition, thus, considering sociodemographic factors of study population is necessary when investigating long-term health effects of SGA. Rapid postnatal catch-up growth in SGA infants is related to a number of obesity-related metabolic disorders. Contrary, persistent poor postnatal growth is associated with more frequent infection, short stature, and impaired cognitive development [17]. It has been suggested that the optimal growth trajectory for SGA may be fast catch-up growth to about the 30th percentile in the first several months, with modest catch-up growth thereafter, to around the 50th percentile by 7 years old [221]. Establishing the optimal growth patterns in SGA to minimize short- and long-term adverse health risks is important, and further studies will be needed. Timing of adrenarche, frequency of clinical manifestations, and onset of puberty seem to be altered in children born SGA, although studies have produced conflicting results. These alternations could affect intrauterine growth and development predispose individuals and leading to early onset of hypertension, chronic kidney disease and endothelial, vascular, and

metabolic abnormalities [222]. Epigenetic modifications, which occur in children born SGA, are thought to underlie part of the metabolic programming that subsequently effects somatic and pubertal development [223]. According to the literature, four of the five earlier studies found a positive association between CD and being born SGA in subsets of study participants with CD, while the other study did not find the association [224]. The biological mechanism underlying an association between SGA and CD remains unclear. Several possible pathways have been suggested including changed cell-mediated immunological development which may affect intestinal immunity, different feeding practices, or greater susceptibility to infection in children born SGA than other children [224].

2.5 Summary

Some of the environmental contaminants have been suggested to play a role in adverse birth outcomes and many studies have shown a dose-dependent manner. From individual studies and meta-analysis, systematic reviews of recent years, evidences on outdoor air pollution and PCB exposures during pregnancy, and adverse birth outcomes have been strengthened. Especially, fairly good evidence for maternal exposure to $PM_{2.5}$ pollution during pregnancy is associated with increased risk of adverse birth outcomes. Heavy metal exposures and PFOS and PFOA exposure during pregnancy may moderately be associated with adverse birth outcomes; however, not sufficient investigation has been conducted for substitutions for PFOS and PFOA. Inconsistent findings have been reported for phthalates and BPA exposure during pregnancy and birth outcomes. These environmental chemicals have short half-lives and thus, exposure assessment at one time point only reflects recent exposure and there might be exposure misclassification. This may contribute to disagreement among study findings. These short half-lives chemicals are ubiquitous and detected from majority of the pregnant women, and thus further studies with accurate exposure assessment are warranted. In addition, according to the fetal origin of adult diseases hypothesis, the intrauterine environment through developmental plasticity may permanently influence long-term health and disease; health issues should be continuously investigated through adulthood. Some of the studies mentioned different influence between male and female infants or sex-specific of environmental exposure on birth outcomes. Further studies are necessary to elucidate mechanisms of observed results from epidemiological studies. PTB and SGA are associated with adverse metabolic and cardiovascular health in adulthood; however, the effect of outcomes on birth weight varies. PTB and SGA are also associated with increased accretion of fat in the period before or immediately after birth. The excess neonatal fat accretion is a key to understand the pathway that leads to adverse metabolic and cardiovascular health in later life. For studies aiming to understand the origins of obesity and metabolic syndrome, assessment of neonatal body composition should also be considered (Tables 2.1 and 2.2).

Table 2.1 Environmental risk factors on adverse birth outcomes

Exposures	Miscarriage/stillbirth	Ref.	Preterm birth	Ref.	Low birth weight/ reduced birth weight	Ref.	Small for gestational age	Ref.
Outdoor air pollution (PM, CO, SO_2, NO_2, PAHs, O_3)	Consistent finding of negative association	[20–28]	A number of studies reported an increased risk	[29–34, 36–40]	Studies reported an increased risk, however, O_3, SO_2, and heavy metals provided less consistent findings	[37, 41–56]	A number of studies reported negative association	[37, 57]
Heavy metals (Cd, As, Hg)	High level exposure and chronic exposure (As, Pb, Hg) are reported to be associated	[61–64]	High level exposure to Pb is reported to be associated with an increased risk. Findings on other heavy metal exposures are inconsistent	[47, 67–76]	Cd, Pb exposures are reported to be associated with reduced birth weight. Findings on Hg are inconsistent	[47, 77–87]	Findings based on birth cohort have been controversial. Consumption of certain nutrients may have protective effects on heavy metal exposure and possibly a reason for inconsistent findings	[56, 88–93]
Organochlorine compounds	DDT, DDE exposure is reported to be associated with an increased risk Accidental exposure to PCBs is associated with an increased risk.	[95, 99–110]	Studies investigated high level exposure to DDT reported an increased risk. Findings on HCB exposure are inconclusive	[105, 106, 111–122]	Both reduced and increased birth weight in association with exposure to DDT, DDE have been reported. PCBs, HCH exposure provides less conclusive findings. Gene–environmental interaction has been suggested	[48, 92, 94, 111, 119–133]	Only limited number of studies were reported. Not sufficient evidence	[135–137]

(continued)

Table 2.1 (continued)

Exposures	Miscarriage/stillbirth	Ref.	Preterm birth	Ref.	Low birth weight/ reduced birth weight	Ref.	Small for gestational age	Ref.
Per- and Polyfluoroalkyl substances (PFAS)	Not sufficient number of studies have been reported		Findings on PFOS, PFOA exposures were inconclusive	[138–143]	Consistent finding on PFOA exposure and reduced birth weight. Findings on PFOS and other PFAS exposures are inconsistent DNA methylation as a mediator has been reported	[144–150]	Finding on PFOA exposure is moderately consistent	[135, 136]
Polybrominated diphenyl esters (PBDEs)	Not sufficient number of studies have been reported		Suggestive increased risk has been reported; however, not sufficient number of studies have been conducted	[151]	Inconsistent findings have been reported	[152, 153]	Not sufficient number of studies have been reported	
Organophosphate pesticides and pyrethroid	Not sufficient number of studies have been reported		Only limited number of studies reported small increased risk	[154–156]	Occupational exposure may be associated with reduced birth weight	[155, 157–163]	Not sufficient number of studies have been reported	
Phthalates	Some evidence on increased risk have been reported	[167, 168]	Inconsistent findings have been reported	[169–174]	Reduced birth weight has been reported	[175–178]	Based on limited number of studies, some of the metabolites have been suggested to be associated with an increased risk	[180]
Bisphenol A	Limited number of studies have been conducted and not sufficient evidence is provided	[183, 184]	Based on small number of studies, inconsistent findings have been reported	[185–188]	Inconsistent findings have been reported	[189–195]	Not sufficient number of studies have been reported	[190]

Table 2.2 Possible mode of action and mechanism of environmental exposure on adverse birth outcomes

Exposures	Mode of action and mechanism	Reference
Outdoor air pollution (PM, CO, SO_2, NO_2, PAHs, O_3)	Increasing systematic oxidative stress and inflammation Impairing placentation Causing endocrine disruption Increasing maternal susceptibility to infections Causing allergic immune response and reduction in heart rate variability	[33, 57, 58]
Heavy metals	Affecting fertilized egg or zygote implantation Accumulating in placental transfer cells and causing a decrease in uterine blood flow and decreasing transfer of nutrients to the fetus	[87]
Organochlorine compounds	Declining sperm counts Increasing in time to conception Intrauterine growth restriction Acting as estrogen agonist or antagonist, having androgenic properties	[88, 90, 91]
Per- and polyfluoroalkyl substances (PFAS)	Hormone disruption Altering lipid metabolism Immunotoxicity in pregnant women Direct fetal toxicity	[87]
Phthalates	Interacting with PPAR with decrease in aromatase activity Hormonal mimicry	[152, 153]
Bisphenol A	Increasing in expression of estrogen-regulated genes Impacting endocrine signaling by downregulating expression of ERα and PR Impacting endometrial angiogenesis by downregulating vascular endothelial growth factor expression	[165, 166]

Acknowledgements This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development(AMED) under Grant Number JP18gk0110032.

References

1. Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. Lancet. 1986;1(8489):1077–81.
2. Sutton P, et al. Toxic environmental chemicals: the role of reproductive health professionals in preventing harmful exposures. Am J Obstet Gynecol. 2012;207(3):164–73.
3. Stillerman KP, et al. Environmental exposures and adverse pregnancy outcomes: a review of the science. Reprod Sci. 2008;15(7):631–50.
4. Windham G, Fenster L. Environmental contaminants and pregnancy outcomes. Fertil Steril. 2008;89(2 Suppl):e111–6.

5. Kishi RA, Miyashita A, Kobayashi C, Miura S, Minatoya RM. The Hokkaido study on environment and children's health. In: Sata FF, Hanson HM, editors. Pre-emptive medicine: public health aspects of developmental origins of health and disease. Singapore: Springer; 2019.
6. Kishi R, et al. The Hokkaido Birth Cohort Study on environment and children's health: cohort profile-updated 2017. Environ Health Prev Med. 2017;22(1):46.
7. Practice Committee of the American Society for Reproductive Medicine. Evaluation and treatment of recurrent pregnancy loss: a committee opinion. Fertil Steril. 2012;98(5):1103–11.
8. Krieg SA, Shahine LK, Lathi RB. Environmental exposure to endocrine-disrupting chemicals and miscarriage. Fertil Steril. 2016;106(4):941–7.
9. de Bernis L, et al. Stillbirths: ending preventable deaths by 2030. Lancet. 2016;387(10019):703–16.
10. Zheng D, et al. Factors associated with spontaneous abortion: a cross-sectional study of Chinese populations. Reprod Health. 2017;14(1):33.
11. Blencowe H, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162–72.
12. Savitz DA, Murnane P. Behavioral influences on preterm birth: a review. Epidemiology. 2010;21(3):291–9.
13. OECD. CO1.3: Low birth weight, L.a.S.A. Social Policy Division-Directorate of Employment, Editor. 2018. https://doi.org/oecdkorea.org/resource/download/2017/eng/CO_1_3_Low_birth_weight.pdf
14. de Onis M, Habicht JP. Anthropometric reference data for international use: recommendations from a World Health Organization Expert Committee. Am J Clin Nutr. 1996;64(4):650–8.
15. WHO. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1995;854:1–452.
16. Ding G, et al. Application of a global reference for fetal-weight and birthweight percentiles in predicting infant mortality. BJOG. 2013;120(13):1613–21.
17. Saenger P, et al. Small for gestational age: short stature and beyond. Endocr Rev. 2007;28(2):219–51.
18. Clayton PE, et al. Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. J Clin Endocrinol Metab. 2007;92(3):804–10.
19. Zeve D, et al. Small at birth, but how small? The definition of SGA revisited. Horm Res Paediatr. 2016;86(5):357–60.
20. Ebisu K, et al. Cause-specific stillbirth and exposure to chemical constituents and sources of fine particulate matter. Environ Res. 2018;160:358–64.
21. Zang H, et al. Ambient air pollution and the risk of stillbirth: a population-based prospective birth cohort study in the coastal area of China. Environ Sci Pollut Res Int. 2019;26(7):6717–24.
22. Yang S, et al. Ambient air pollution the risk of stillbirth: a prospective birth cohort study in Wuhan, China. Int J Hyg Environ Health. 2018;221(3):502–9.
23. DeFranco E, et al. Air pollution and stillbirth risk: exposure to airborne particulate matter during pregnancy is associated with fetal death. PLoS One. 2015;10(3):e0120594.
24. Faiz AS, et al. Does ambient air pollution trigger stillbirth? Epidemiology. 2013;24(4):538–44.
25. Li Z, et al. Impact of ambient PM2.5 on adverse birth outcome and potential molecular mechanism. Ecotoxicol Environ Saf. 2019;169:248–54.
26. Pope DP, et al. Risk of low birth weight and stillbirth associated with indoor air pollution from solid fuel use in developing countries. Epidemiol Rev. 2010;32:70–81.
27. Aminu M, et al. Causes of and factors associated with stillbirth in low- and middle-income countries: a systematic literature review. BJOG. 2014;121(Suppl 4):141–53.
28. Blencowe H, et al. National, regional, and worldwide estimates of stillbirth rates in 2015, with trends from 2000: a systematic analysis. Lancet Glob Health. 2016;4(2):e98–e108.
29. Trasande L, Malecha P, Attina TM. Particulate matter exposure and preterm birth: estimates of U.S. attributable burden and economic costs. Environ Health Perspect. 2016;124(12):1913–8.

30. Malley CS, et al. Preterm birth associated with maternal fine particulate matter exposure: a global, regional and national assessment. Environ Int. 2017;101:173–82.
31. Guo T, et al. The association between ambient PM2.5 exposure and the risk of preterm birth in China: a retrospective cohort study. Sci Total Environ. 2018;633:1453–9.
32. Rappazzo KM, et al. Exposure to elemental carbon, organic carbon, nitrate, and sulfate fractions of fine particulate matter and risk of preterm birth in New Jersey, Ohio, and Pennsylvania (2000–2005). Environ Health Perspect. 2015;123(10):1059–65.
33. Laurent O, et al. A statewide nested case-control study of preterm birth and air pollution by source and composition: California, 2001-2008. Environ Health Perspect. 2016;124(9):1479–86.
34. Basu R, et al. Association between PM2.5 and PM2.5 constituents and preterm delivery in California, 2000-2006. Paediatr Perinat Epidemiol. 2017;31(5):424–34.
35. Slama R, et al. Meeting report: atmospheric pollution and human reproduction. Environ Health Perspect. 2008;116(6):791–8.
36. Sapkota A, et al. Exposure to particulate matter and adverse birth outcomes: a comprehensive review and meta-analysis. Air Qual Atmos Health. 2012;5(4):369–81.
37. Shah PS, Balkhair T. Air pollution and birth outcomes: a systematic review. Environ Int. 2011;37(2):498–516.
38. Liu C, et al. Different exposure levels of fine particulate matter and preterm birth: a meta-analysis based on cohort studies. Environ Sci Pollut Res Int. 2017;24(22):17976–84.
39. Singh VK, et al. Comparison of polycyclic aromatic hydrocarbon levels in placental tissues of Indian women with full- and preterm deliveries. Int J Hyg Environ Health. 2008;211(5-6):639–47.
40. Wilhelm M, et al. Traffic-related air toxics and preterm birth: a population-based case-control study in Los Angeles County, California. Environ Health. 2011;10:89.
41. Li X, et al. Association between ambient fine particulate matter and preterm birth or term low birth weight: an updated systematic review and meta-analysis. Environ Pollut. 2017;227:596–605.
42. Dadvand P, et al. Maternal exposure to particulate air pollution and term birth weight: a multi-country evaluation of effect and heterogeneity. Environ Health Perspect. 2013;121(3):267–373.
43. Pedersen M, et al. Ambient air pollution and low birthweight: a European cohort study (ESCAPE). Lancet Respir Med. 2013;1(9):695–704.
44. Dedele A, Grazuleviciene R, Miskinyte A. Individual exposure to nitrogen dioxide and adverse pregnancy outcomes in Kaunas study. Int J Environ Health Res. 2017;27(3):230–40.
45. Coker E, et al. Multi-pollutant exposure profiles associated with term low birth weight in Los Angeles County. Environ Int. 2016;91:1–13.
46. Stieb DM, et al. Ambient air pollution, birth weight and preterm birth: a systematic review and meta-analysis. Environ Res. 2012;117:100–11.
47. Zhu M, et al. Maternal low-level lead exposure and fetal growth. Environ Health Perspect. 2010;118(10):1471–5.
48. Vrijheid M, et al. Environmental pollutants and child health-a review of recent concerns. Int J Hyg Environ Health. 2016;219(4-5):331–42.
49. Bloom MS, et al. Maternal arsenic exposure and birth outcomes: a comprehensive review of the epidemiologic literature focused on drinking water. Int J Hyg Environ Health. 2014;217(7):709–19.
50. Milton AH, et al. A review of the effects of chronic arsenic exposure on adverse pregnancy outcomes. Int J Environ Res Public Health. 2017;14:6.
51. McDermott S, et al. Systematic review of chromium and nickel exposure during pregnancy and impact on child outcomes. J Toxicol Environ Health A. 2015;78(21-22):1348–68.
52. Eum JH, et al. Maternal blood manganese level and birth weight: a MOCEH birth cohort study. Environ Health. 2014;13(1):31.
53. Zota AR, et al. Maternal blood manganese levels and infant birth weight. Epidemiology. 2009;20(3):367–73.
54. Xia W, et al. A case-control study of maternal exposure to chromium and infant low birth weight in China. Chemosphere. 2016;144:1484–9.

55. Sun X, et al. Association between prenatal nickel exposure and preterm low birth weight: possible effect of selenium. Environ Sci Pollut Res Int. 2018;25(26):25888–95.
56. Hu J, et al. Association of adverse birth outcomes with prenatal exposure to vanadium: a population-based cohort study. Lancet Planet Health. 2017;1(6):e230–41.
57. Winckelmans E, et al. Fetal growth and maternal exposure to particulate air pollution--more marked effects at lower exposure and modification by gestational duration. Environ Res. 2015;140:611–8.
58. Huo X, et al. Maternal urinary metabolites of PAHs and its association with adverse birth outcomes in an intensive e-waste recycling area. Environ Pollut. 2019;245:453–61.
59. van den Hooven EH, et al. Chronic air pollution exposure during pregnancy and maternal and fetal C-reactive protein levels: the Generation R Study. Environ Health Perspect. 2012;120(5):746–51.
60. Hall KC, Robinson JC. Association between maternal exposure to pollutant particulate matter 2.5 and congenital heart defects: a systematic review. JBI Database System Rev Implement Rep. 2019;17:1695–716.
61. Rahman A, Kumarathasan P, Gomes J. Infant and mother related outcomes from exposure to metals with endocrine disrupting properties during pregnancy. Sci Total Environ. 2016;569-570:1022–31.
62. Quansah R, et al. Association of arsenic with adverse pregnancy outcomes/infant mortality: a systematic review and meta-analysis. Environ Health Perspect. 2015;123(5):412–21.
63. Yorifuji T, et al. Temporal trends of infant and birth outcomes in Minamata after severe methylmercury exposure. Environ Pollut. 2017;231(Pt 2):1586–92.
64. Itai Y, et al. An epidemiological study of the incidence of abnormal pregnancy in areas heavily contaminated with methylmercury. Environ Sci. 2004;11(2):83–97.
65. Yorifuji T, Kashima S. Secondary sex ratio in regions severely exposed to methylmercury "Minamata disease". Int Arch Occup Environ Health. 2016;89(4):659–65.
66. Ferguson KK, O'Neill MS, Meeker JD. Environmental contaminant exposures and preterm birth: a comprehensive review. J Toxicol Environ Health B Crit Rev. 2013;16(2):69–113.
67. Andrews KW, Savitz DA, Hertz-Picciotto I. Prenatal lead exposure in relation to gestational age and birth weight: a review of epidemiologic studies. Am J Ind Med. 1994;26(1):13–32.
68. Sowers M, et al. Blood lead concentrations and pregnancy outcomes. Arch Environ Health. 2002;57(5):489–95.
69. Cantonwine D, et al. Critical windows of fetal lead exposure: adverse impacts on length of gestation and risk of premature delivery. J Occup Environ Med. 2010;52(11):1106–11.
70. Vigeh M, et al. Blood lead at currently acceptable levels may cause preterm labour. Occup Environ Med. 2011;68(3):231–4.
71. Landgren O. Environmental pollution and delivery outcome in southern Sweden: a study with central registries. Acta Paediatr. 1996;85(11):1361–4.
72. Zhang YL, et al. Effect of environmental exposure to cadmium on pregnancy outcome and fetal growth: a study on healthy pregnant women in China. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2004;39(9):2507–15.
73. Ahmad SA, et al. Arsenic in drinking water and pregnancy outcomes. Environ Health Perspect. 2001;109(6):629–31.
74. Yang CY, et al. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. Environ Res. 2003;91(1):29–34.
75. Myers SL, et al. Maternal drinking water arsenic exposure and perinatal outcomes in inner Mongolia, China. J Epidemiol Community Health. 2010;64(4):325–9.
76. Xue F, et al. Maternal fish consumption, mercury levels, and risk of preterm delivery. Environ Health Perspect. 2007;115(1):42–7.
77. Sun H, et al. The effects of prenatal exposure to low-level cadmium, lead and selenium on birth outcomes. Chemosphere. 2014;108:33–9.
78. Salpietro CD, et al. Cadmium concentration in maternal and cord blood and infant birth weight: a study on healthy non-smoking women. J Perinat Med. 2002;30(5):395–9.

79. Irgens A, et al. Reproductive outcome in offspring of parents occupationally exposed to lead in Norway. Am J Ind Med. 1998;34(5):431–7.
80. Berkowitz Z, et al. Lead exposure and birth outcomes in five communities in Shoshone County, Idaho. Int J Hyg Environ Health. 2006;209(2):123–32.
81. Gundacker C, et al. Perinatal lead and mercury exposure in Austria. Sci Total Environ. 2010;408(23):5744–9.
82. Garcia-Esquinas E, et al. Lead, mercury and cadmium in umbilical cord blood and its association with parental epidemiological variables and birth factors. BMC Public Health. 2013;13:841.
83. Drouillet-Pinard P, et al. Prenatal mercury contamination: relationship with maternal seafood consumption during pregnancy and fetal growth in the 'EDEN mother-child' cohort. Br J Nutr. 2010;104(8):1096–100.
84. Ding G, et al. Prenatal low-level mercury exposure and neonatal anthropometry in rural northern China. Chemosphere. 2013;92(9):1085–9.
85. Lee BE, et al. Interaction between GSTM1/GSTT1 polymorphism and blood mercury on birth weight. Environ Health Perspect. 2010;118(3):437–43.
86. Burch JB, et al. Mercury in fish and adverse reproductive outcomes: results from South Carolina. Int J Health Geogr. 2014;13:30.
87. Vejrup K, et al. Prenatal mercury exposure and infant birth weight in the Norwegian Mother and Child Cohort Study. Public Health Nutr. 2014;17(9):2071–80.
88. Johnston JE, et al. Maternal cadmium levels during pregnancy associated with lower birth weight in infants in a North Carolina cohort. PLoS One. 2014;9(10):e109661.
89. Yang J, et al. Maternal urinary cadmium concentrations in relation to preterm birth in the Healthy Baby Cohort Study in China. Environ Int. 2016;94:300–6.
90. Liu H, et al. Maternal arsenic exposure and birth outcomes: a birth cohort study in Wuhan, China. Environ Pollut. 2018;236:817–23.
91. Kobayashi S, et al. Association of blood mercury levels during pregnancy with infant birth size by blood selenium levels in the Japan Environment and Children's Study: a prospective birth cohort. Environ Int. 2019;125:418–29.
92. Miyashita C, et al. Effects of in utero exposure to polychlorinated biphenyls, methylmercury, and polyunsaturated fatty acids on birth size. Sci Total Environ. 2015;533:256–65.
93. Jelliffe-Pawlowski LL, et al. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. J Perinatol. 2006;26(3):154–62.
94. Zheng T, et al. Effects of environmental exposures on fetal and childhood growth trajectories. Ann Glob Health. 2016;82(1):41–99.
95. Beard J. DDT and human health. Sci Total Environ. 2006;355(1-3):78–89.
96. Kumar S. Occupational, environmental and lifestyle factors associated with spontaneous abortion. Reprod Sci. 2011;18(10):915–30.
97. Carlsen E, et al. Evidence for decreasing quality of semen during past 50 years. BMJ. 1992;305(6854):609–13.
98. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet. 1993;341(8857):1392–5.
99. Korrick SA, et al. Association of DDT with spontaneous abortion: a case-control study. Ann Epidemiol. 2001;11(7):491–6.
100. Venners SA, et al. Preconception serum DDT and pregnancy loss: a prospective study using a biomarker of pregnancy. Am J Epidemiol. 2005;162(8):709–16.
101. Longnecker MP, et al. Maternal serum level of the DDT metabolite DDE in relation to fetal loss in previous pregnancies. Environ Res. 2005;97(2):127–33.
102. Tsukimori K, et al. Long-term effects of polychlorinated biphenyls and dioxins on pregnancy outcomes in women affected by the Yusho incident. Environ Health Perspect. 2008;116(5):626–30.
103. Schnorr TM, et al. Spontaneous abortion, sex ratio, and paternal occupational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Environ Health Perspect. 2001;109(11):1127–32.

104. Pan X, et al. Association between environmental dioxin-related toxicants exposure and adverse pregnancy outcome: systematic review and meta-analysis. Int J Fertil Steril. 2015;8(4):351–66.
105. Eskenazi B, et al. Maternal serum dioxin levels and birth outcomes in women of Seveso, Italy. Environ Health Perspect. 2003;111(7):947–53.
106. Le TN, Johansson A. Impact of chemical warfare with agent orange on women's reproductive lives in Vietnam: a pilot study. Reprod Health Matters. 2001;9(18):156–64.
107. Yorifuji T, et al. Regional impact of exposure to a polychlorinated biphenyl and polychlorinated dibenzofuran mixture from contaminated rice oil on stillbirth rate and secondary sex ratio. Environ Int. 2013;59:12–5.
108. Qu Y, et al. Risk factors of stillbirth in rural China: a national cohort study. Sci Rep. 2019;9(1):365.
109. Razi S, et al. Exposure to pistachio pesticides and stillbirth: a case-control study. Epidemiol Health. 2016;38:e2016016.
110. Roncati L, Piscioli F, Pusiol T. The endocrine disruptors among the environmental risk factors for stillbirth. Sci Total Environ. 2016;563-564:1086–7.
111. Longnecker MP, et al. Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet. 2001;358(9276):110–4.
112. Bergonzi R, et al. Persistent organochlorine compounds in fetal and maternal tissues: evaluation of their potential influence on several indicators of fetal growth and health. Sci Total Environ. 2011;409(15):2888–93.
113. Ribas-Fito N, et al. Association of hexachlorobenzene and other organochlorine compounds with anthropometric measures at birth. Pediatr Res. 2002;52(2):163–7.
114. Fenster L, et al. Association of in utero organochlorine pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect. 2006;114(4):597–602.
115. Torres-Arreola L, et al. Preterm birth in relation to maternal organochlorine serum levels. Ann Epidemiol. 2003;13(3):158–62.
116. Saxena MC, et al. Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature of full-term delivery. J Anal Toxicol. 1981;5(1):6–9.
117. Pathak R, et al. Maternal and cord blood levels of organochlorine pesticides: association with preterm labor. Clin Biochem. 2009;42(7-8):746–9.
118. Wassermann M, et al. Premature delivery and organochlorine compounds: polychlorinated biphenyls and some organochlorine insecticides. Environ Res. 1982;28(1):106–12.
119. Longnecker MP, et al. Maternal levels of polychlorinated biphenyls in relation to preterm and small-for-gestational-age birth. Epidemiology. 2005;16(5):641–7.
120. Wojtyniak BJ, et al. Association of maternal serum concentrations of 2,2', 4,4'5,5'-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p'-DDE) levels with birth weight, gestational age and preterm births in Inuit and European populations. Environ Health. 2010;9:56.
121. Govarts E, et al. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European Birth Cohorts. Environ Health Perspect. 2012;120(2):162–70.
122. Revich B, et al. Dioxin exposure and public health in Chapaevsk, Russia. Chemosphere. 2001;43(4-7):951–66.
123. Weisskopf MG, et al. Maternal exposure to Great Lakes sport-caught fish and dichlorodiphenyl dichloroethylene, but not polychlorinated biphenyls, is associated with reduced birth weight. Environ Res. 2005;97(2):149–62.
124. Siddiqui MK, et al. Persistent chlorinated pesticides and intra-uterine foetal growth retardation: a possible association. Int Arch Occup Environ Health. 2003;76(1):75–80.
125. Konishi K, et al. Prenatal exposure to PCDDs/PCDFs and dioxin-like PCBs in relation to birth weight. Environ Res. 2009;109(7):906–13.

126. Kobayashi S, et al. Genetic association of aromatic hydrocarbon receptor (AHR) and cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) polymorphisms with dioxin blood concentrations among pregnant Japanese women. Toxicol Lett. 2013;219(3):269–78.
127. Kobayashi S, et al. Dioxin-metabolizing genes in relation to effects of prenatal dioxin levels and reduced birth size: The Hokkaido study. Reprod Toxicol. 2017;67:111–6.
128. Karmaus W, Zhu X. Maternal concentration of polychlorinated biphenyls and dichlorodiphenyl dichlorethylene and birth weight in Michigan fish eaters: a cohort study. Environ Health. 2004;3(1):1.
129. Gladen BC, et al. Persistent organochlorine compounds and birth weight. Ann Epidemiol. 2003;13(3):151–7.
130. Farhang L, et al. Association of DDT and DDE with birth weight and length of gestation in the child health and development studies, 1959-1967. Am J Epidemiol. 2005;162(8):717–25.
131. Tan J, et al. Exposure to persistent organic pollutants in utero and related maternal characteristics on birth outcomes: a multivariate data analysis approach. Chemosphere. 2009;74(3):428–33.
132. Murphy LE, et al. Maternal serum preconception polychlorinated biphenyl concentrations and infant birth weight. Environ Health Perspect. 2010;118(2):297–302.
133. Sonneborn D, et al. Prenatal polychlorinated biphenyl exposures in eastern Slovakia modify effects of social factors on birthweight. Paediatr Perinat Epidemiol. 2008;22(3):202–13.
134. Kobayashi S, et al. Gender-specific association of exposure to non-dioxin-like polychlorinated biphenyls during pregnancy with methylation levels of H19 and long interspersed nuclear element-1 in cord blood in the Hokkaido study. Toxicology. 2017;390:135–45.
135. Govarts E, et al. Prenatal exposure to endocrine disrupting chemicals and risk of being born small for gestational age: pooled analysis of seven European birth cohorts. Environ Int. 2018;115:267–78.
136. Lauritzen HB, et al. Maternal serum levels of perfluoroalkyl substances and organochlorines and indices of fetal growth: a Scandinavian case-cohort study. Pediatr Res. 2017;81(1):33–42.
137. Chand S, et al. CYP17A1 gene polymorphisms and environmental exposure to organochlorine pesticides contribute to the risk of small for gestational age. Eur J Obstet Gynecol Reprod Biol. 2014;180:100–5.
138. Apelberg BJ, et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect. 2007;115(11):1670–6.
139. Savitz DA, et al. Relationship of perfluorooctanoic acid exposure to pregnancy outcome based on birth records in the mid-Ohio Valley. Environ Health Perspect. 2012;120(8):1201–7.
140. Meng Q, et al. Prenatal exposure to perfluoroalkyl substances and birth outcomes; an updated analysis from the Danish National Birth Cohort. Int J Environ Res Public Health. 2018;15(9):E1832.
141. Whitworth KW, et al. Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. Am J Epidemiol. 2012;175(12):1209–16.
142. Chen MH, et al. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS One. 2012;7(8):e42474.
143. Wu K, et al. Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environ Int. 2012;48:1–8.
144. Johnson PI, et al. The Navigation Guide - evidence-based medicine meets environmental health: systematic review of human evidence for PFOA effects on fetal growth. Environ Health Perspect. 2014;122(10):1028–39.
145. Bach CC, et al. Perfluoroalkyl and polyfluoroalkyl substances and human fetal growth: a systematic review. Crit Rev Toxicol. 2015;45(1):53–67.
146. Kobayashi S, et al. Effects of prenatal perfluoroalkyl acid exposure on cord blood IGF2/H19 methylation and ponderal index: The Hokkaido Study. J Expo Sci Environ Epidemiol. 2017;27(3):251–9.

147. Washino N, et al. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect. 2009;117(4):660–7.
148. Minatoya M, et al. Association of prenatal exposure to perfluoroalkyl substances with cord blood adipokines and birth size: The Hokkaido Study on environment and children's health. Environ Res. 2017;156:175–82.
149. Tsai MS, et al. Determinants and temporal trends of perfluoroalkyl substances in pregnant women: The Hokkaido Study on Environment and Children's Health. Int J Environ Res Public Health. 2018;15(5):E989.
150. Okada E, et al. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011. Environ Int. 2013;60:89–96.
151. Wu K, et al. Polybrominated diphenyl ethers in umbilical cord blood and relevant factors in neonates from Guiyu, China. Environ Sci Technol. 2010;44(2):813–9.
152. Lignell S, et al. Prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) may influence birth weight among infants in a Swedish cohort with background exposure: a cross-sectional study. Environ Health. 2013;12:44.
153. Chao HR, et al. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. Environ Int. 2007;33(2):239–45.
154. Eskenazi B, et al. Association of in utero organophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect. 2004;112(10):1116–24.
155. Sathyanarayana S, et al. Maternal pesticide use and birth weight in the agricultural health study. J Agromedicine. 2010;15(2):127–36.
156. Ling C, et al. Prenatal exposure to ambient pesticides and preterm birth and term low birthweight in agricultural regions of California. Toxics. 2018;6(3):E41.
157. Figa-Talamanca I. Occupational risk factors and reproductive health of women. Occup Med. 2006;56(8):521–31.
158. Rauch SA, et al. Associations of prenatal exposure to organophosphate pesticide metabolites with gestational age and birth weight. Environ Health Perspect. 2012;120(7):1055–60.
159. Larsen AE, Gaines SD, Deschenes O. Agricultural pesticide use and adverse birth outcomes in the San Joaquin Valley of California. Nat Commun. 2017;8(1):302.
160. Hanke W, et al. The use of pesticides in a Polish rural population and its effect on birth weight. Int Arch Occup Environ Health. 2003;76(8):614–20.
161. Saillenfait AM, Ndiaye D, Sabate JP. Pyrethroids: exposure and health effects--an update. Int J Hyg Environ Health. 2015;218(3):281–92.
162. Mytton OT, et al. Safety of benzyl benzoate lotion and permethrin in pregnancy: a retrospective matched cohort study. BJOG. 2007;114(5):582–7.
163. Kennedy D, et al. Pregnancy outcome following exposure to permethrin and use of teratogen information. Am J Perinatol. 2005;22(2):87–90.
164. Zhang J, et al. Prenatal pyrethroid insecticide exposure and thyroid hormone levels and birth sizes of neonates. Sci Total Environ. 2014;488-489:275–9.
165. Lovekamp TN, Davis BJ. Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. Toxicol Appl Pharmacol. 2001;172(3):217–24.
166. Lovekamp-Swan T, Davis BJ. Mechanisms of phthalate ester toxicity in the female reproductive system. Environ Health Perspect. 2003;111(2):139–45.
167. Toft G, et al. Association between pregnancy loss and urinary phthalate levels around the time of conception. Environ Health Perspect. 2012;120(3):458–63.
168. Mu D, et al. Levels of phthalate metabolites in urine of pregnant women and risk of clinical pregnancy loss. Environ Sci Technol. 2015;49(17):10651–7.
169. Huang Y, et al. Phthalate levels in cord blood are associated with preterm delivery and fetal growth parameters in Chinese women. PLoS One. 2014;9(2):e87430.
170. Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. JAMA Pediatr. 2014;168(1):61–7.

171. Ferguson KK, et al. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. Environ Int. 2014;70:118–24.
172. Ferguson KK, et al. Mediation of the relationship between maternal phthalate exposure and preterm birth by oxidative stress with repeated measurements across pregnancy. Environ Health Perspect. 2017;125(3):488–94.
173. Meeker JD, et al. Urinary phthalate metabolites in relation to preterm birth in Mexico city. Environ Health Perspect. 2009;117(10):1587–92.
174. Adibi JJ, et al. Maternal urinary metabolites of Di-(2-Ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. Am J Epidemiol. 2009;169(8):1015–24.
175. Lenters V, et al. Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environ Health Perspect. 2016;124(3):365–72.
176. Ashley-Martin J, et al. A birth cohort study to investigate the association between prenatal phthalate and bisphenol A exposures and fetal markers of metabolic dysfunction. Environ Health. 2014;13:84.
177. Minatoya M, et al. Prenatal di-2-ethylhexyl phthalate exposure and cord blood adipokine levels and birth size: The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2017;579:606–11.
178. Minatoya M, et al. Association between prenatal bisphenol A and phthalate exposures and fetal metabolic related biomarkers: The Hokkaido Study on Environment and Children's Health. Environ Res. 2018;161:505–11.
179. Wassenaar PNH, Legler J. Systematic review and meta-analysis of early life exposure to di(2-ethylhexyl) phthalate and obesity related outcomes in rodents. Chemosphere. 2017;188:174–81.
180. Bloom MS, et al. Racial disparity in maternal phthalates exposure; association with racial disparity in fetal growth and birth outcomes. Environ Int. 2019;127:473–86.
181. Varayoud J, et al. Long-lasting effects of neonatal bisphenol A exposure on the implantation process. Vitam Horm. 2014;94:253–75.
182. Bosquiazzo VL, et al. Effects of neonatal exposure to bisphenol A on steroid regulation of vascular endothelial growth factor expression and endothelial cell proliferation in the adult rat uterus. Biol Reprod. 2010;82(1):86–95.
183. Lathi RB, et al. Conjugated bisphenol A in maternal serum in relation to miscarriage risk. Fertil Steril. 2014;102(1):123–8.
184. Shen Y, et al. Higher urinary bisphenol A concentration is associated with unexplained recurrent miscarriage risk: evidence from a case-control study in eastern China. PLoS One. 2015;10(5):e0127886.
185. Cantonwine D, et al. Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study. Environ Health. 2010;9:62.
186. Cantonwine DE, et al. Urinary bisphenol A levels during pregnancy and risk of preterm birth. Environ Health Perspect. 2015;123(9):895–901.
187. Behnia F, et al. High bisphenol A (BPA) concentration in the maternal, but not fetal, compartment increases the risk of spontaneous preterm delivery. J Matern Fetal Neonatal Med. 2016;29(22):3583–9.
188. Patel CJ, et al. Investigation of maternal environmental exposures in association with self-reported preterm birth. Reprod Toxicol. 2014;45:1–7.
189. Miao M, et al. In utero exposure to bisphenol-A and its effect on birth weight of offspring. Reprod Toxicol. 2011;32(1):64–8.
190. Chou WC, et al. Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environ Health. 2011;10:94.
191. Huo W, et al. Maternal urinary bisphenol A levels and infant low birth weight: a nested case-control study of the Health Baby Cohort in China. Environ Int. 2015;85:96–103.

192. Lee BE, et al. Prenatal bisphenol A and birth outcomes: MOCEH (Mothers and Children's Environmental Health) study. Int J Hyg Environ Health. 2014;217(2-3):328–34.
193. Philippat C, et al. Exposure to phthalates and phenols during pregnancy and offspring size at birth. Environ Health Perspect. 2012;120(3):464–70.
194. Padmanabhan V, et al. Maternal bisphenol-A levels at delivery: a looming problem? J Perinatol. 2008;28(4):258–63.
195. Wolff MS, et al. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect. 2008;116(8):1092–7.
196. Slama R, et al. Epidemiologic tools to study the influence of environmental factors on fecundity and pregnancy-related outcomes. Epidemiol Rev. 2014;36:148–64.
197. Irving RJ, et al. Adult cardiovascular risk factors in premature babies. Lancet. 2000;355(9221):2135–6.
198. de Jong M, Cranendonk A, van Weissenbruch MM. Components of the metabolic syndrome in early childhood in very-low-birth-weight infants and term small and appropriate for gestational age infants. Pediatr Res. 2015;78(4):457–61.
199. Hofman PL, et al. Premature birth and later insulin resistance. N Engl J Med. 2004;351(21):2179–86.
200. Bellou V, et al. Risk factors for type 2 diabetes mellitus: an exposure-wide umbrella review of meta-analyses. PLoS One. 2018;13(3):e0194127.
201. Rogers LK, Velten M. Maternal inflammation, growth retardation, and preterm birth: insights into adult cardiovascular disease. Life Sci. 2011;89(13-14):417–21.
202. Roggero P, et al. Is term newborn body composition being achieved postnatally in preterm infants? Early Hum Dev. 2009;85(6):349–52.
203. Johnson MJ, et al. Preterm birth and body composition at term equivalent age: a systematic review and meta-analysis. Pediatrics. 2012;130(3):e640–9.
204. Ratnasingham A, et al. Review: is rapid fat accumulation in early life associated with adverse later health outcomes? Placenta. 2017;54:125–30.
205. Low Birth Weight and Nephron Number Working Group. The impact of kidney development on the life course: a consensus document for action. Nephron. 2017;136(1):3–49.
206. Korzeniewski SJ, et al. Neurodevelopment at age 10 years of children born <28 weeks with fetal growth restriction. Pediatrics. 2017;140(5):e20170697.
207. Arthursson PSH, et al. Atypical neuronal activation during a spatial working memory task in 13-year-old very preterm children. Hum Brain Mapp. 2017;38(12):6172–84.
208. Neubauer V, et al. Bronchopulmonary dysplasia is associated with delayed structural brain maturation in preterm infants. Neonatology. 2015;107(3):179–84.
209. Linsell L, et al. Prognostic factors for behavioral problems and psychiatric disorders in children born very preterm or very low birth weight: a systematic review. J Dev Behav Pediatr. 2016;37(1):88–102.
210. Linsell L, et al. Prognostic factors for poor cognitive development in children born very preterm or with very low birth weight: a systematic review. JAMA Pediatr. 2015;169(12):1162–72.
211. JAMA Patient Page. Low birth weight. JAMA. 2002;287(2):270.
212. Kelishadi R, et al. Low birthweight or rapid catch-up growth: which is more associated with cardiovascular disease and its risk factors in later life? A systematic review and cryptanalysis. Paediatr Int Child Health. 2015;35(2):110–23.
213. Rasyid H, Bakri S. Intra-uterine growth retardation and development of hypertension. Acta Med Indones. 2016;48(4):320–4.
214. Belbasis L, et al. Birth weight in relation to health and disease in later life: an umbrella review of systematic reviews and meta-analyses. BMC Med. 2016;14(1):147.
215. Saad NJ, et al. Birth weight and lung function in adulthood: a systematic review and meta-analysis. Ann Am Thorac Soc. 2017;14(6):994–1004.
216. Rosenberg A. The IUGR newborn. Semin Perinatol. 2008;32(3):219–24.
217. Roth CL, Sathyanarayana S. Mechanisms affecting neuroendocrine and epigenetic regulation of body weight and onset of puberty: potential implications in the child born small for gestational age (SGA). Rev Endocr Metab Disord. 2012;13(2):129–40.

218. Yajnik CS. The lifecycle effects of nutrition and body size on adult adiposity, diabetes and cardiovascular disease. Obes Rev. 2002;3(3):217–24.
219. Ibanez L, et al. Low body adiposity and high leptinemia in breast-fed infants born small-for-gestational-age. J Pediatr. 2010;156(1):145–7.
220. Ibanez L, et al. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91(6):2153–8.
221. Lei X, et al. The optimal postnatal growth trajectory for term small for gestational age babies: a prospective cohort study. J Pediatr. 2015;166(1):54–8.
222. Mericq V, et al. Long-term metabolic risk among children born premature or small for gestational age. Nat Rev Endocrinol. 2017;13(1):50–62.
223. Roth CL, DiVall S. Consequences of early life programing by genetic and environmental influences: a synthesis regarding pubertal timing. Endocr Dev. 2016;29:134–52.
224. Wingren CJ, Agardh D, Merlo J. Revisiting the risk of celiac disease in children born small for gestational age: a sibling design perspective. Scand J Gastroenterol. 2012;47(6):632–9.

Chapter 3
Effects of Environmental Chemical Exposure on Birth Defects (Except Cryptorchidism and Hypospadias)

Tomoyuki Hanaoka, Chihiro Miyashita, Kumiko Itoh, and Reiko Kishi

Abstract Currently, the causes of birth defects remain unknown in approximately 80% of the cases. Here, the etiologies are likely multifactorial and may involve the genetic background, exposure to drugs, environmental chemical exposure, infections, maternal factors, and intrauterine mechanical factors. In this review, we discuss the effects of environmental chemical exposure on the incidence of birth defects by summarizing the previous epidemiological studies. Notably, chemical exposure was most frequently associated with elevated risks of central nervous system and congenital heart defects and oral clefts than with other types of birth defects. Although exposure to air pollutants, persistent organic pollutants, polycyclic aromatic hydrocarbons, and perfluorinated compounds were associated with increased risks, no substance-specific birth defects were identified. Many case–control studies had the limitation due to poor exposure assessment. In terms of the risk assessment, it is difficult that epidemiological study indicates the hazard identification including the dose-response relationship. We conclude that descriptions of the disease prevalence and individual chemical exposure levels are important roles of reproductive epidemiological study.

Keywords Birth defects · Air pollutants · Persistent organic pollutants · Polycyclic aromatic hydrocarbons · Perfluorinated compounds

T. Hanaoka · C. Miyashita · R. Kishi (✉)
Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Hokkaido, Japan
e-mail: rkishi@med.hokudai.ac.jp

K. Itoh
Department of Nursing, Faculty of Health Science, Hokkaido University of Science, Sapporo, Hokkaido, Japan

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_3

3.1 Introduction

Experimental evidence has demonstrated the complexity and multilayered nature of fetal development, as even a single defective mechanism will result in birth defects. The twenty-first century marks the era of elucidation of the mechanisms underlying fetal development at a molecular level. Soon, it may be possible to use an in vitro molecular disruption in this process to screen comprehensively for the risk of birth defects caused by exposure to environmental chemicals. However, many previous epidemiological studies of the relationship between exposure to environmental chemicals and birth defects have been limited by small sample sizes or weak exposure assessments.

This review summarizes current trends in the prevalence of birth defects, the possible mechanisms underlying birth defects associated with environmental chemical exposure, and previous epidemiological findings regarding this topic. This paper also discusses the goals and expectations of future epidemiological studies that aim to investigate the causative agents of birth defects.

3.2 Trends in the Prevalence of Birth Defects

Table 3.1 summarizes data from populations in Japan and Texas, USA that were obtained from the International Clearinghouse for Birth Defects Surveillance and Research (ICBDSR) [1, 2]. In this table, the Japanese data are hospital-based, the US data are population-based, and both datasets use the total number of live births and stillbirths as the denominator. In this table, all diseases have been classified according to the method described by St. Louis et al. [3]. In this comparison, Japan reported a relatively higher incidence of congenital heart defects, oral cleft, gastrointestinal defects, and chromosomal anomalies but a lower incidence of genitourinary

Table 3.1 Prevalence rates of birth defects in Japan and Texas as reported by the ICBDSR

	Japan		Texas, USA	
Birth year	2005	2012	2004	2011
Live births and stillbirths	71,765	108,087	380,905	377,336
Central nervous system	7.2	6.4	7.3	8.1
Ear defects	1.3	2.0	3.3	3.6
Congenital heart defects	19.1	22.7	13.4	17.2
Oral cleft	24.7	28.6	16.6	17.2
Gastrointestinal defects	22.2	17.5	7.6	13.5
Genitourinary defects	4.6	5.7	16.3	17.4
Musculoskeletal defects	22.2	19.3	17.1	21.5
Chromosomal anomalies	21.9	29.3	16.4	18.7

Rates are presented as numbers per 10,000 live births and stillbirths
Diseases are classified according to the method described by St. Louis et al. [3]

defects (hypospadias). However, these datasets cannot be compared directly because of the differences in the maximum age at diagnosis and the criteria used to define stillbirth.

The analysis reveals an increasing incidence of oral clefts and chromosomal anomalies over time in Japan, but not in Texas. This difference appears to be based on an environmental difference, which includes factors related to maternal exposure, rather than racial differences between these two populations. This observation highlights the need to conduct a focused study of disease in which the incidence changes over time and among regions.

3.3 Causes of Birth Defects

Figure 3.1 presents the causes of birth defects according to Feldkamp et al. [4]. The causes are unknown in approximately 80% of cases, whereas birth defects caused by obvious teratogens account for only 0.8%. Additionally, chromosomal abnormalities and birth defects of unknown etiology are likely attributable to multifactorial causal factors, which include the genetic background, drugs, environmental chemical exposure, infection, maternal factors, and intrauterine mechanical factors. However, each factor likely makes a small individual contribution; and therefore, it is difficult to assess the risks associated with environmental chemical exposure in the context of an epidemiological study. Many researchers believe that although the majority of teratogenic factors have a threshold below which no malformations are induced (i.e., the "no-effect" level), a sufficiently high dose of xenobiotics will affect a developing embryo [5]. Therefore, it is important to determine whether daily exposure to a particular environmental chemical will have an effect on fetal development.

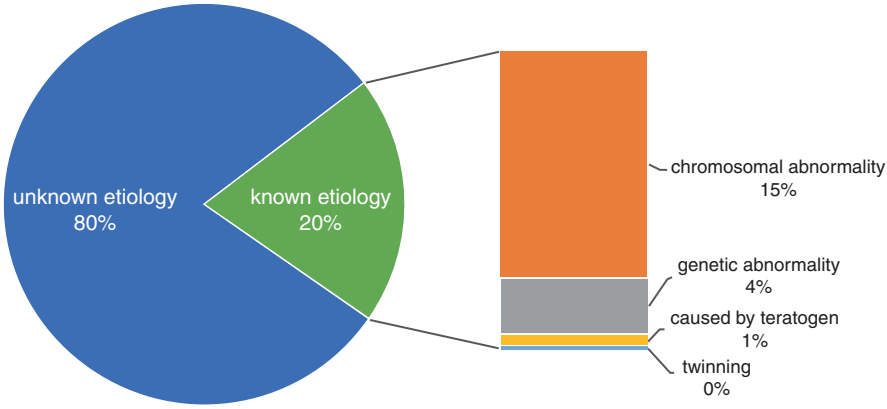


Fig. 3.1 Etiology of birth defects [4]

3.4 Mechanisms Associated with Birth Defects

The complex intracellular signal transduction mechanisms associated with normal development and embryonic induction have largely been elucidated. Specifically, the roles of factors such as intercellular communication factors, morphogens, receptor tyrosine kinases, the Notch-Delta pathway, various transcription factors, and epigenetic factors have been identified [6]. Exposure to an environmental chemical can disrupt these complex molecular pathways within the cell, and exposure beyond the "no-effect" threshold can lead to irreversible disruption and birth defects.

According to Levi, the initiating mechanisms of birth defects include mutations, chromosomal abnormalities, interference with mitosis and/or nucleic acid function, nutritional deficiencies, changes or deficiency in the energy supply, changes in osmolarity, changes in the cell membrane, and enzyme inhibition [5]. Exposure to a causative substance can trigger these mechanisms, which alters molecular signaling pathways within the cell and thus causes birth defects.

Epigenetic alterations of the germ cells can lead to inherited phenotypes. Although researchers have concluded that the malformations caused by thalidomide are not inherited [7], recent studies have demonstrated the heritability of cleft palate [8]. This latter finding suggests that cleft palate may be attributable to an epigenetic change. Table 3.2 presents the chemicals and pollutants that are known to induce methylation [9]. DNA methylation is a known epigenetic factor. In some cases, exposure to these substances may induce an epigenetic alteration that leads to a birth defect.

In addition to genotoxic and endocrine-disrupting effects, chemicals such as tetrachlorodibenzo-p-dioxin (TCDD) and halogenated aromatic hydrocarbons can directly affect molecular signaling within the cell. These chemicals activate the aryl hydrocarbon receptor (AHR) and trigger downstream cell signaling pathways associated with extracellular matrix synthesis and repair. Additionally, AHR activation is known to affect the regulation of processes essential for development, including cell cycle progression, proliferation, differentiation, apoptosis, and cell migration [10–14]. Studies have shown that intrauterine exposure to TCDD and

Table 3.2 Chemicals and pollutants known to induce methylation [9]

Tobacco smoke
Particulate air pollution
Asbestos
Bisphenol A (BPA)
Diethylstilbestrol (DES)
Metal ions (such as chromium, cadmium, nickel, arsenic, and methylmercury)
Vinclozolin
Methoxychlor
Silica
Benzene
Di- and trichloroacetic acid, trichloroethylene

halogenated aromatic hydrocarbons induces isolated clefts of the secondary palate and hydronephrosis even at doses that do not cause other toxicities in adult women during pregnancy or breastfeeding. Accordingly, the effects of low-level exposure to these chemicals cannot be ignored [15–17].

3.5 Exposure to High Levels of Environmental Chemicals and Birth Defects

Several historical incidents have demonstrated the causal association between exposure to high levels of environmental chemicals and birth defects. For example, the epidemic of Minamata disease in the Kumamoto prefecture of Japan in the 1950s is a famous example. This central nervous system disorder is caused by exposure to organic mercury via polluted water. In this case, the children of exposed mothers developed congenital Minamata disease, which manifested as microcephaly in 60% of cases [18]. In another example, a fivefold increase in the incidence of hydrocephalus was observed among the children of Vietnam War veterans who had been exposed to Agent Orange (odds ratio [OR] 5.1, 95% confidence interval [CI] 1.1–23.1) [19]. Notably, these cases may not be suitable as references for risk evaluations because they involved exposure to high doses of xenobiotic chemicals, which are always likely to affect embryonic or fetal development.

3.6 Exposure to Low Levels of Environmental Chemicals and Birth Defects

3.6.1 Dioxins, Dioxin-Like Compounds, and Pesticides

A nested case–control study conducted in the USA reported a high incidence of L-transposition of the great arteries (OR 13.4, 95% CI 4.7–37.8) in the Baltimore–Washington region, which was attributed to exposure to industrial pollution and hazardous waste [20]. In an Italian cohort study, the levels of exposure to dioxins were estimated based on the distance between the participants' dwellings and incinerators, as well as the atmospheric concentration of dioxins. However, that study did not identify an increased risk of birth defects [21]. Similarly, a Japanese cross-sectional study did not identify a significant correlation between the distance from an incinerator and the risk of birth defects [22]. In France, a population-based case–control study estimated the dioxin exposure level based on the distance between the participants' dwellings and waste processing plants. Interestingly, that study reported an increased risk of urinary tract birth defects in the offspring of women exposed to dioxin levels at or above the median atmospheric level during early pregnancy (OR 2.0, 95% CI 1.2–3.4 for atmospheric dioxins) [23].

Finally, a case–control study in the USA observed no significantly elevated risk of spina bifida upon pesticide exposure, which was estimated on the basis of occupational history. However, the effects of specific agricultural chemicals were unknown [24].

3.6.2 Perfluorinated Compounds

Animal studies have reported an association between fetal exposure to perfluorinated compounds (PFASs) and an increased risk of left ventricular hypertrophy [25]. The C8 Health Project, a cohort study in the USA, observed a significant increase in the risk of brain defects with each interquartile increase in the estimated serum perfluorooctanoate (PFOA) exposure of pregnant women in regions where the drinking water was contaminated with high concentrations of PFOA [26]. However, another cross-sectional study in the USA found no correlation between the residential area, as classified by public water supply category, and the risk of birth defects [27]. Similarly, a nested case–control study of 215 male infants in Denmark and Finland found no correlation between the level of PFASs in cord blood and cryptorchidism [28].

3.6.3 Organic Solvents

In Canada, a prospective study that compared 125 pregnant women with occupational exposure to organic solvents and a group of pregnant women without such exposure identified an increased risk of major malformations in the former group (risk ratio (RR) 13.0, 95% CI 1.8–99.5) [29]. By contrast, a register-based prospective study conducted in Russia between 1973 and 2005 did not observe statistically significant increases in the risks of multiple, circulatory system, genital organ, or musculoskeletal system anomalies in female employees at nickel-refining plants who were exposed occupationally to organic solvents [30]. As exposure assessments of in occupational populations are relatively accurate, further studies are expected.

3.6.4 Air Pollutants

A meta-analysis of 10 epidemiological studies conducted in the USA, UK, Australia, Korea, Taiwan, and other countries found that prenatal exposure to nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) was associated with an increased risk of tetralogy of

Fallot (NO_2: OR 1.2, 95% CI 1.02–1.4 and SO_2: OR 1.03, 95% CI 1.01–1.1), while exposure to fine particulate matter (PM10) was associated with an increased risk of atrial septal defect (OR 1.1, 95% CI 1.01–1.3). However, no correlations were identified between air pollutants and other birth defects [31]. In Italy, a case–control study observed a correlation between SO_2 exposure and congenital heart disease (OR 3.2, 95% CI 1.4–7.3) [32], while a population-based case–control study identified a borderline dose-response relationship between PM10 exposure and musculoskeletal and chromosomal abnormalities but not cardiovascular defects [33]. In summary, many previous studies have observed associations between air pollutants and birth defects. However, these studies have been limited by the difficulties inherent to individual exposure assessments.

3.6.5 Nitro Compounds

Several studies have suggested a relationship between exposure to nitro compounds in drinking water and birth defects such as neural tube defects (NTDs) [34–36], general central nervous system defects [37], oral cleft defects, musculoskeletal defects [36], and congenital heart defects [38]. A case–control study conducted by the US National Birth Defects Prevention Study estimated the intake of nitrates from drinking water and found that prenatal exposure to this factor correlated with an increased risk of limb deficiency (OR 1.8, 95% CI 1.1–3.1), cleft palate (OR 1.9, 95% CI 1.2–3.1), and cleft lip (OR 1.8, 95% CI 1.1–3.1) [34]. In future studies, exposure assessments will likely be based on the internal doses.

3.6.6 Summary of Previous Epidemiological Studies

Table 3.3 summarizes the statistically significant associations identified in previous epidemiological studies. Although not all of these studies focused solely on birth defects, the risks of central nervous system and congenital heart defects and oral clefts in response to chemical exposure were reported more frequently than were other types of birth defects. Exposure to air pollutants, persistent organic pollutants, polycyclic aromatic hydrocarbons, and PFOA led to increased risks. Interestingly, no substance-specific birth defects were identified, suggesting that different substances affect the same developmental mechanisms and/or the same substances affect different developmental mechanisms. Notably, the gestational age of 6 weeks is considered the most sensitive period for the development of all three organ types; and therefore, study outcomes may be affected by the accurate assessment of exposure during that period (Fig. 3.2).

Table 3.3 Previously reported significant relationships of birth defects with environmental chemical exposure

Birth defects	Environmental chemicals	Study design	Individual exposure assessment	References
Central nervous system	CO	Case–control		[40]
	NO	Case–control		[40]
	NO_2	Case–control		[40]
	Amide, benzimidazole, methyl carbamate, organophosphorus pesticides	Case–control		[41]
	Avermectin, petroleum derivative, bromoynil	Case–control		[42]
	PCBs and PBDEs	Case–control	Available	[43]
	PAHs, *o*,*p*-DDT, *c*-HCH, and *a*-Endosulfun	Case–control	Available	[44]
	PAH	Case–control		[45]
		Case–control	Available	[46]
	PFOA	Case–control	Available	[26]
Congenital heart defects	CO	Case–control		[47]
		Case–control		[48]
	NO_2	Case–control		[49]
	O_3	Case–control		[50]
		Case–control		[47]
	SO_2	Case–control		[48]
		Case–control		[32]
	PM <10 μm	Case–control		[51]
		Case–control		[48]
		Case–control		[51]
		Case–control		[52]
	PM <2.5 μm	Case–control		[49]
		Case–control		[51]
	Chlorophenoxy herbicide	Case–control		[53]
Oral cleft	O3	Case–control		[54]
		Case–control		[55]
	SO_2	Case–control		[50]
	PM <2.5 μm	Case–control		[55]
	Atrazine	Birth cohort		[56]
	2,6-dinitroanaline, dithiocarbamate MITC, 2,6-dinitroanaline, dithiocarbamate MITC	Case–control		[42]
	Herbicides, rodenticides	Cross-sectional		[57]

Table 3.3 (continued)

Birth defects	Environmental chemicals	Study design	Individual exposure assessment	References
Gastrointestinal defects	PM <10 μm	Case–control		[58]
	Pesticides except for atrazine	Birth cohort		[56]
	Atrazine	Case–control		[59]
	Herbicides, insecticides	Case–control		[60]
Musculoskeletal defects	Atrazine and nitrate	Observational		[61]
	Atrazine	Birth cohort		[56]
	Pesticides except for atrazine	Birth cohort		[56]
	Herbicides, rodenticides	Cross-sectional		[57]
Chromosomal anomalies	Atrazine	Birth cohort		[56]

Previous studies in which the specific diseases or chemicals were unknown are not included in this table

3.7 Future Epidemiological Studies

The importance of surveillance is unquestionable. However, current large-scale, multicenter surveillance methods are prone to errors and limitations [39]. The interpretation of changes in exposure over time and the utilization of these data in epidemiological studies should be addressed in future studies, which may require a narrowed focus on diseases with an increased incidence.

Currently, there are two possible approaches to the use of genetic polymorphism data in future epidemiological studies. One approach involves the consideration of gene-environment interactions if genetic information involving developmental processes (e.g., AhR polymorphisms) is available. The other approach involves the consideration of polymorphisms in phase I and phase II metabolic enzymes when evaluating the accumulation of lipophilic chemicals in the body.

Finally, an accurate risk assessment depends on an accurate exposure assessment. Therefore, descriptive studies of the individual exposure level are important. The effects of socioeconomic factors on the incidence of birth defects related to chemical exposure can only be clarified through an epidemiological analysis.

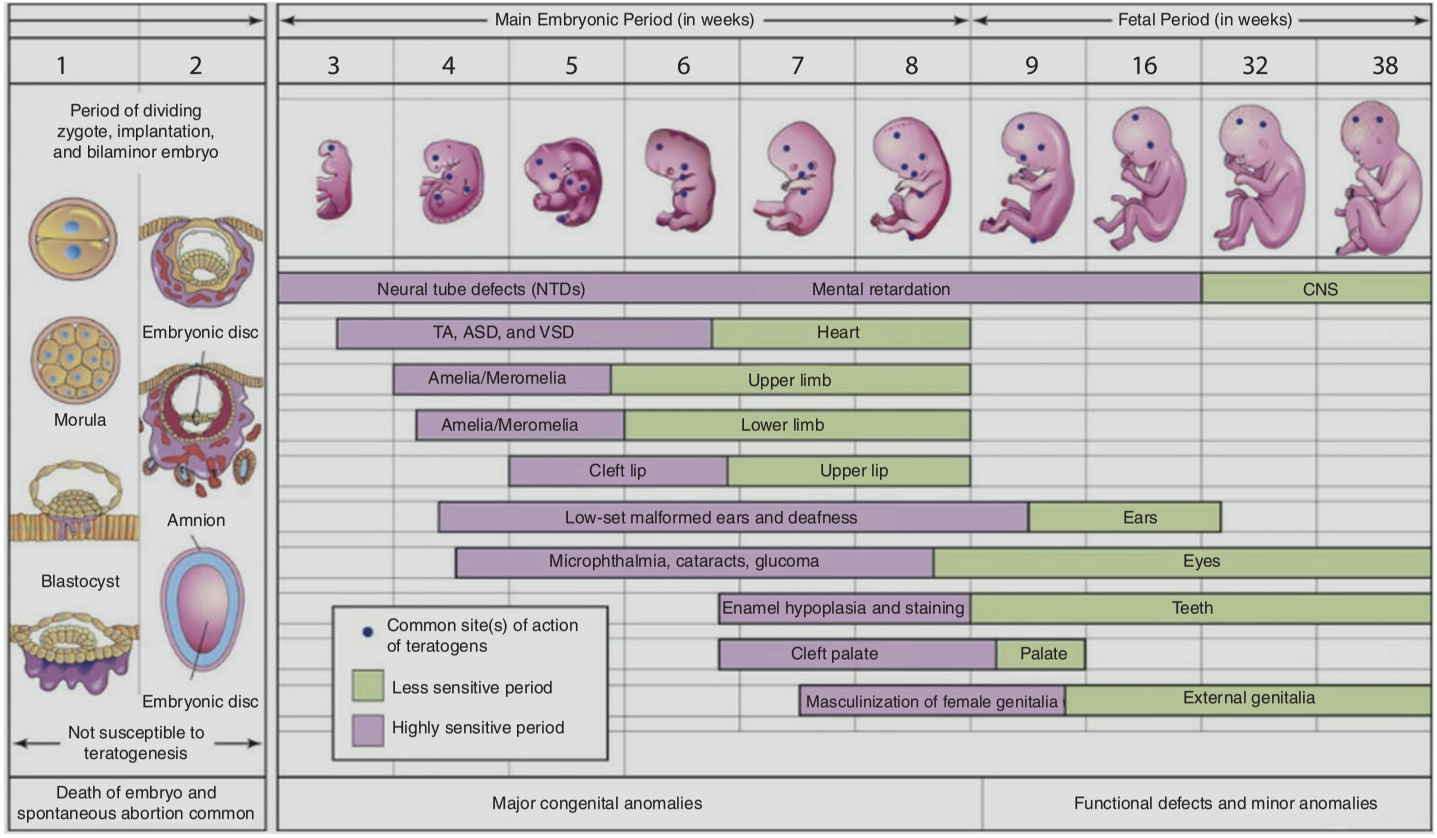


Fig. 3.2 Critical periods in human prenatal development [6]

3.8 Conclusions

We review previous epidemiological studies concerning environmental chemical exposure and birth defects. Many case–control studies had the limitation due to poor exposure assessment. Even previous large-scale prospective studies, i.e., the Norwegian cohort study, showed ambiguous results due to small sample sizes. In terms of the risk assessment, it is difficult that epidemiological study indicates the hazard identification including the dose-response relationship. Descriptions of the disease prevalence and individual chemical exposure levels are important roles of reproductive epidemiological study.

Acknowledgements This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18gk0110032.

References

1. ICBDSR. Annual report 2007. The Centre of the International Clearinghouse for Birth Defects Surveillance and Research, Roma, Italy; 2007.
2. ICBDSR. Annual report 2014. The Centre of the International Clearinghouse for Birth Defects Surveillance and Research, Roma, Italy; 2014.
3. St Louis AM, Kim K, Browne ML, Liu G, Liberman RF, Nembhard WN, Canfield MA, Copeland G, Fornoff J, Kirby RS, National Birth Defects Prevention N. Prevalence trends of selected major birth defects: a multi-state population-based retrospective study, United States, 1999 to 2007. Birth Defects Res. 2017;109(18):1442–50. https://doi.org/10.1002/bdr2.1113.
4. Feldkamp ML, Carey JC, Byrne JLB, Krikov S, Botto LD. Etiology and clinical presentation of birth defects: population based study. BMJ. 2017;357:j2249. https://doi.org/10.1136/bmj.j2249.
5. Levi P. Principles and mechanisms of teratogenesis. In: Kob V, editor. Teratogens: chemicals which cause birth defects. Amsterdam: Elsevier Science; 1993. p. 1–19.
6. Moore K, Persaud T, Torchia M. Essentials of embryology and birth defects. Philadelphia: Elsevier Health Sciences; 2016.
7. Smithells D. Does thalidomide cause second generation birth defects? Drug Saf. 1998;19(5):339–41. https://doi.org/10.2165/00002018-199819050-00001.
8. Jamilian A, Sarkarat F, Jafari M, Neshandar M, Amini E, Khosravi S, Ghassemi A. Family history and risk factors for cleft lip and palate patients and their associated anomalies. Stomatologija. 2017;19(3):78–83.
9. Feil R, Fraga MF. Epigenetics and the environment: emerging patterns and implications. Nat Rev Genet. 2012;13(2):97–109. https://doi.org/10.1038/nrg3142.
10. Gomez-Duran A, Carvajal-Gonzalez JM, Mulero-Navarro S, Santiago-Josefat B, Puga A, Fernandez-Salguero PM. Fitting a xenobiotic receptor into cell homeostasis: how the dioxin receptor interacts with TGFbeta signaling. Biochem Pharmacol. 2009;77(4):700–12. https://doi.org/10.1016/j.bcp.2008.08.032.
11. Haarmann-Stemmann T, Bothe H, Abel J. Growth factors, cytokines and their receptors as downstream targets of arylhydrocarbon receptor (AhR) signaling pathways. Biochem Pharmacol. 2009;77(4):508–20. https://doi.org/10.1016/j.bcp.2008.09.013.
12. Kung T, Murphy KA, White LA. The aryl hydrocarbon receptor (AhR) pathway as a regulatory pathway for cell adhesion and matrix metabolism. Biochem Pharmacol. 2009;77(4):536–46. https://doi.org/10.1016/j.bcp.2008.09.031.

13. Puga A, Ma C, Marlowe JL. The aryl hydrocarbon receptor cross-talks with multiple signal transduction pathways. Biochem Pharmacol. 2009;77(4):713–22. https://doi.org/10.1016/j.bcp.2008.08.031.
14. Ma C, Marlowe JL, Puga A. The aryl hydrocarbon receptor at the crossroads of multiple signaling pathways. EXS. 2009;99:231–57.
15. Birnbaum LS, Weber H, Harris MW, Lamb JC, McKinney JD. Toxic interaction of specific polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin: increased incidence of cleft palate in mice. Toxicol Appl Pharmacol. 1985;77(2):292–302.
16. Courtney KD, Moore JA. Teratology studies with 2,4,5-trichlorophenoxyacetic acid and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicol Appl Pharmacol. 1971;20(3):396–403.
17. Neubert D, Zens P, Rothenwallner A, Merker HJ. A survey of the embryotoxic effects of TCDD in mammalian species. Environ Health Perspect. 1973;5:67–79. https://doi.org/10.1289/ehp.730567.
18. Harada M. Congenital minamata disease: intrauterine methylmercury poisoning. Teratology. 1978;18(2):285–8. https://doi.org/10.1002/tera.1420180216.
19. CtRtHEiVVoEt H. Veterans and agent orange: update 1996. Washington, DC: The National Academy Press; 1996.
20. Kuehl KS, Loffredo CA. Population-based study of l-transposition of the great arteries: possible associations with environmental factors. Birth Defects Res A Clin Mol Teratol. 2003;67(3):162–7. https://doi.org/10.1002/bdra.10015.
21. Vinceti M, Malagoli C, Werler MM, Filippini T, De Girolamo G, Ghermandi G, Fabbi S, Astolfi G, Teggi S. Adverse pregnancy outcomes in women with changing patterns of exposure to the emissions of a municipal waste incinerator. Environ Res. 2018;164:444–51. https://doi.org/10.1016/j.envres.2018.03.024.
22. Tango T, Fujita T, Tanihata T, Minowa M, Doi Y, Kato N, Kunikane S, Uchiyama I, Tanaka M, Uehata T. Risk of adverse reproductive outcomes associated with proximity to municipal solid waste incinerators with high dioxin emission levels in Japan. J Epidemiol. 2004;14(3):83–93.
23. Cordier S, Lehebel A, Amar E, Anzivino-Viricel L, Hours M, Monfort C, Chevrier C, Chiron M, Robert-Gnansia E. Maternal residence near municipal waste incinerators and the risk of urinary tract birth defects. Occup Environ Med. 2010;67(7):493–9. https://doi.org/10.1136/oem.2009.052456.
24. Pettigrew SM, Bell EM, Van Zutphen AR, Rocheleau CM, Shaw GM, Romitti PA, Olshan A, Lupo PJ, Soim A, Makelarski JA, Michalski AM, Sanderson W, The National Birth Defects Prevention Study. Paternal and joint parental occupational pesticide exposure and spina bifida in the National Birth Defects Prevention Study, 1997 to 2002. Birth Defects Res A Clin Mol Teratol. 2016;106(11):963–71. https://doi.org/10.1002/bdra.23551.
25. Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, Butenhoff JL, Stevenson LA, Lau C. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse I: maternal and prenatal evaluations. Toxicol Sci. 2003;74(2):369–81. https://doi.org/10.1093/toxsci/kfg121.
26. Stein CR, Savitz DA, Elston B, Thorpe PG, Gilboa SM. Perfluorooctanoate exposure and major birth defects. Reprod Toxicol. 2014;47:15–20. https://doi.org/10.1016/j.reprotox.2014.04.006.
27. Nolan LA, Nolan JM, Shofer FS, Rodway NV, Emmett EA. Congenital anomalies, labor/delivery complications, maternal risk factors and their relationship with perfluorooctanoic acid (PFOA)-contaminated public drinking water. Reprod Toxicol. 2010;29(2):147–55. https://doi.org/10.1016/j.reprotox.2009.10.012.
28. Vesterholm Jensen D, Christensen J, Virtanen HE, Skakkebaek NE, Main KM, Toppari J, Veje CW, Andersson AM, Nielsen F, Grandjean P, Jensen TK. No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland. Reproduction. 2014;147(4):411–7. https://doi.org/10.1530/REP-13-0444.
29. Khattak S, Moghtader G, McMartin K, Barrera M, Kennedy D, Koren G. Pregnancy outcome following gestational exposure to organic solvents: a prospective controlled study. JAMA. 1999;281(12):1106–9.

30. Vaktskjold A, Talykova LV, Nieboer E. Congenital anomalies in newborns to women employed in jobs with frequent exposure to organic solvents--a register-based prospective study. BMC Pregnancy Childbirth. 2011;11:83. https://doi.org/10.1186/1471-2393-11-83.
31. Vrijheid M, Martinez D, Manzanares S, Dadvand P, Schembari A, Rankin J, Nieuwenhuijsen M. Ambient air pollution and risk of congenital anomalies: a systematic review and meta-analysis. Environ Health Perspect. 2011;119(5):598–606. https://doi.org/10.1289/ehp.1002946.
32. Gianicolo EA, Mangia C, Cervino M, Bruni A, Andreassi MG, Latini G. Congenital anomalies among live births in a high environmental risk area--a case-control study in Brindisi (southern Italy). Environ Res. 2014;128:9–14. https://doi.org/10.1016/j.envres.2013.11.002.
33. Vinceti M, Malagoli C, Malavolti M, Cherubini A, Maffeis G, Rodolfi R, Heck JE, Astolfi G, Calzolari E, Nicolini F. Does maternal exposure to benzene and PM10 during pregnancy increase the risk of congenital anomalies? A population-based case-control study. Sci Total Environ. 2016;541:444–50. https://doi.org/10.1016/j.scitotenv.2015.09.051.
34. Brender JD, Olive JM, Felkner M, Suarez L, Marckwardt W, Hendricks KA. Dietary nitrites and nitrates, nitrosatable drugs, and neural tube defects. Epidemiology. 2004;15(3):330–6.
35. Croen LA, Todoroff K, Shaw GM. Maternal exposure to nitrate from drinking water and diet and risk for neural tube defects. Am J Epidemiol. 2001;153(4):325–31.
36. Dorsch MM, Scragg RK, McMichael AJ, Baghurst PA, Dyer KF. Congenital malformations and maternal drinking water supply in rural South Australia: a case-control study. Am J Epidemiol. 1984;119(4):473–86.
37. Arbuckle TE, Sherman GJ, Corey PN, Walters D, Lo B. Water nitrates and CNS birth defects: a population-based case-control study. Arch Environ Health. 1988;43(2):162–7. https://doi.org/10.1080/00039896.1988.9935846.
38. Cedergren MI, Selbing AJ, Lofman O, Kallen BA. Chlorination byproducts and nitrate in drinking water and risk for congenital cardiac defects. Environ Res. 2002;89(2):124–30.
39. Hanaoka T, Tamura N, Ito K, Sasaki S, Araki A, Ikeno T, Miyashita C, Ito S, Minakami H, Cho K, Endo T, Baba T, Miyamoto T, Sengoku K, Kishi R, Hokkaido Study on Environment and Children's Health. Prevalence and risk of birth defects observed in a prospective cohort study: The Hokkaido Study on Environment and Children's Health. J Epidemiol. 2018;28(3):125–32. https://doi.org/10.2188/jea.JE20160108.
40. Padula AM, Tager IB, Carmichael SL, Hammond SK, Lurmann F, Shaw GM. The association of ambient air pollution and traffic exposures with selected congenital anomalies in the San Joaquin Valley of California. Am J Epidemiol. 2013;177(10):1074–85. https://doi.org/10.1093/aje/kws367.
41. Rull RP, Ritz B, Shaw GM. Neural tube defects and maternal residential proximity to agricultural pesticide applications. Am J Epidemiol. 2006;163(8):743–53. https://doi.org/10.1093/aje/kwj101.
42. Yang W, Carmichael SL, Roberts EM, Kegley SE, Padula AM, English PB, Shaw GM. Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the San Joaquin Valley of California. Am J Epidemiol. 2014;179(6):740–8. https://doi.org/10.1093/aje/kwt324.
43. Ma J, Qiu X, Ren A, Jin L, Zhu T. Using placenta to evaluate the polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) exposure of fetus in a region with high prevalence of neural tube defects. Ecotoxicol Environ Saf. 2012;86:141–6. https://doi.org/10.1016/j.ecoenv.2012.09.005.
44. Ren A, Qiu X, Jin L, Ma J, Li Z, Zhang L, Zhu H, Finnell RH, Zhu T. Association of selected persistent organic pollutants in the placenta with the risk of neural tube defects. Proc Natl Acad Sci U S A. 2011;108(31):12770–5. https://doi.org/10.1073/pnas.1105209108.
45. Li Z, Zhang L, Ye R, Pei L, Liu J, Zheng X, Ren A. Indoor air pollution from coal combustion and the risk of neural tube defects in a rural population in Shanxi Province, China. Am J Epidemiol. 2011;174(4):451–8. https://doi.org/10.1093/aje/kwr108.
46. Naufal Z, Zhiwen L, Zhu L, Zhou GD, McDonald T, He LY, Mitchell L, Ren A, Zhu H, Finnell R, Donnelly KC. Biomarkers of exposure to combustion by-products in a human population

in Shanxi, China. J Expo Sci Environ Epidemiol. 2010;20(4):310–9. https://doi.org/10.1038/jes.2009.19.
47. Ritz B, Yu F, Fruin S, Chapa G, Shaw GM, Harris JA. Ambient air pollution and risk of birth defects in Southern California. Am J Epidemiol. 2002;155(1):17–25.
48. Gilboa SM, Mendola P, Olshan AF, Langlois PH, Savitz DA, Loomis D, Herring AH, Fixler DE. Relation between ambient air quality and selected birth defects, seven county study, Texas, 1997-2000. Am J Epidemiol. 2005;162(3):238–52. https://doi.org/10.1093/aje/kwi189.
49. Stingone JA, Luben TJ, Daniels JL, Fuentes M, Richardson DB, Aylsworth AS, Herring AH, Anderka M, Botto L, Correa A, Gilboa SM, Langlois PH, Mosley B, Shaw GM, Siffel C, Olshan AF, National Birth Defects Prevention Study. Maternal exposure to criteria air pollutants and congenital heart defects in offspring: results from the national birth defects prevention study. Environ Health Perspect. 2014;122(8):863–72. https://doi.org/10.1289/ehp.1307289.
50. Hansen CA, Barnett AG, Jalaludin BB, Morgan GG. Ambient air pollution and birth defects in Brisbane, Australia. PLoS One. 2009;4(4):e5408. https://doi.org/10.1371/journal.pone.0005408.
51. Padula AM, Tager IB, Carmichael SL, Hammond SK, Yang W, Lurmann F, Shaw GM. Ambient air pollution and traffic exposures and congenital heart defects in the San Joaquin Valley of California. Paediatr Perinat Epidemiol. 2013;27(4):329–39. https://doi.org/10.1111/ppe.12055.
52. Agay-Shay K, Friger M, Linn S, Peled A, Amitai Y, Peretz C. Air pollution and congenital heart defects. Environ Res. 2013;124:28–34. https://doi.org/10.1016/j.envres.2013.03.005.
53. Schreinemachers DM. Birth malformations and other adverse perinatal outcomes in four U.S. Wheat-producing states. Environ Health Perspect. 2003;111(9):1259–64. https://doi.org/10.1289/ehp.5830.
54. Hwang BF, Jaakkola JJ. Ozone and other air pollutants and the risk of oral clefts. Environ Health Perspect. 2008;116(10):1411–5. https://doi.org/10.1289/ehp.11311.
55. Marshall EG, Harris G, Wartenberg D. Oral cleft defects and maternal exposure to ambient air pollutants in New Jersey. Birth Defects Res A Clin Mol Teratol. 2010;88(4):205–15. https://doi.org/10.1002/bdra.20650.
56. Winchester PD, Huskins J, Ying J. Agrichemicals in surface water and birth defects in the United States. Acta Paediatr. 2009;98(4):664–9. https://doi.org/10.1111/j.1651-2227.2008.01207.x.
57. Loffredo CA, Silbergeld EK, Ferencz C, Zhang J. Association of transposition of the great arteries in infants with maternal exposures to herbicides and rodenticides. Am J Epidemiol. 2001;153(6):529–36.
58. Padula AM, Tager IB, Carmichael SL, Hammond SK, Yang W, Lurmann FW, Shaw GM. Traffic-related air pollution and selected birth defects in the San Joaquin Valley of California. Birth Defects Res A Clin Mol Teratol. 2013;97(11):730–5. https://doi.org/10.1002/bdra.23175.
59. Waller SA, Paul K, Peterson SE, Hitti JE. Agricultural-related chemical exposures, season of conception, and risk of gastroschisis in Washington State. Am J Obstet Gynecol. 2010;202(3):241–6. https://doi.org/10.1016/j.ajog.2010.01.023.
60. Felix JF, van Dooren MF, Klaassens M, Hop WC, Torfs CP, Tibboel D. Environmental factors in the etiology of esophageal atresia and congenital diaphragmatic hernia: results of a case-control study. Birth Defects Res A Clin Mol Teratol. 2008;82(2):98–105. https://doi.org/10.1002/bdra.20423.
61. Mattix KD, Winchester PD, Scherer LR. Incidence of abdominal wall defects is related to surface water atrazine and nitrate levels. J Pediatr Surg. 2007;42(6):947–9. https://doi.org/10.1016/j.jpedsurg.2007.01.027.

Chapter 4
Cryptorchidism and Hypospadias

Takahiko Mitsui, Fumihiro Sata, and Reiko Kishi

Abstract Several pieces of evidence indicated the effect of environmental chemicals on the prevalence of cryptorchidism and hypospadias. In addition, several other factors, such as genetic factors, gene mutations, endocrinopathies, also cause cryptorchidism and hypospadias. From reviewing papers in this chapter, the evidence for these associations remains controversial; hence, there are not adequate data to establish an association between exposure to environmental chemicals and cryptorchidism or hypospadias. On the other hand, genes and polymorphisms involved in metabolism of environmental endocrine disruptors might influence the risk of male genital malformations. Thus, further cohort studies are required.

Keywords Cryptorchidism · Hypospadias · Endocrine-disrupting chemicals (EDCs) · Environmental endocrine disruptors (EEDs) · Hormone · Gene · Epidemiological study

Abbreviation

AHRs	Aromatic hydrocarbon receptors
ARNT2	Aryl hydrocarbon receptor nuclear translocator 2
BPA	Bisphenol A
CGRP	Calcitonin gene-related peptide
DES	Diethylstilbestrol

T. Mitsui (✉)
Department of Urology, Graduate School of Medical Sciences, University of Yamanashi, Chuo, Yamanashi, Japan
e-mail: tmitsui@yamanashi.ac.jp

F. Sata
Health Center, Chuo University, Tokyo, Japan

R. Kishi
Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Hokkaido, Japan

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_4

DGKK	Diacylglycerol kinase κ
DLCs	Dioxin-like compounds
EDCs	Endocrine-disrupting chemicals
EEDs	Environmental endocrine disruptors
ESR	Estrogen receptor
HCB	Hexachlorobenzene
HCE	Heptachlor epoxide
HOX	Homeobox
INSL3	Insulin-like factor 3
NTSR1	Neurotensin receptor 1
OCPs	Organochlorine pesticides
PBDEs	Polybrominated diphenyl ethers
PCBs	Polychlorinated biphenyls
PFAAs	Perfluoroalkyl acids
PFASs	Perfluoroalkyl substances
PFOA	Perfluorooctanoate
PFOS	Perfluorooctane sulfonate
PRL	Prolactin
TDS	Testicular dysgenesis syndrome

4.1 Introduction

Cryptorchidism and hypospadias are the most common urogenital anomalies. Both conditions have been considered to be linked to the hypothetical testicular dysgenesis syndrome (TDS) [1, 2]. TDS is a result of disrupted embryonic programming and gonadal development during fetal life [1]. The unfavorable environmental factors, such as environmental endocrine disruptors (EEDs), in addition to genetic factors, are considered to be the major etiological factors of TDS. However, the overall pathogenesis of cryptorchidism and hypospadias remains unknown. Moreover, it is most likely multifactorial, involving genetic factors, gene mutations, endocrinopathies, and endocrine disruptors [3, 4].

Exposure to endocrine-disrupting chemicals, even in an occupational environment of parents during gestation, may affect the prevalence of cryptorchidism through changes of the hormonal environment during the prenatal period. This chapter describes the effects of environmental chemicals in addition to genes and polymorphisms on cryptorchidism and hypospadias.

4.2 Cryptorchidism

Cryptorchidism, the most common genital disorder identified at birth is the absence of the testes in the scrotum, and it may induce reduced infertility and an increased risk of testicular cancer. Its rate varied from 1 to 4% in full-term and 1–45% in

preterm male neonates [5]. Several papers reported that the prevalence of cryptorchidism is reported stable or slightly decreasing [6, 7]. Studies have reported that the treatment of cryptorchidism should be recommended between 6 and 12 months of age as it alleviates the semen quality by earlier orchiopexy [8, 9].

It has already been established that two discrete anatomical and hormonal stages are involved in testicular descent. Testicular descent usually occurs prenatally, with the transabdominal phase (the first phase) between 8 and 15 weeks of gestation and the inguinoscrotal phase (the second stage) between 25 and 35 weeks of gestation [10, 11]. The gubernaculum swells to anchor the testes near the groin as the fetus grows, which is regulated by the insulin-like factor 3 (INSL3) in the transabdominal phase [11, 12]. Testosterone makes the cranial suspensory ligament regressed during this phase [11]. In the inguinoscrotal phase, testicular descent occurs when the gubernaculum bulges out of the external ring and migrates to the scrotum. This phase is regulated by androgens and calcitonin gene-related peptide (CGRP) [11]. CGRP, which also regulated by testosterone, is released by the sensory nucleus of the genitofemoral nerve [10, 13–15]. Conclusively, the prenatal environment, particularly sex hormones, regulates testicular descent.

4.3 Hypospadias

Normal development of the urethra and the phallus involves ventral fusion in the proximal to distal direction as a continuous process during 8–14 weeks of gestation [16–18], which were influenced by testosterone and 5-α dihydrotestosterone. These androgens are synthesized in response to a surge of luteinizing hormone from the pituitary. At the end of the third month of the gestation, the urethral folds close over the urethral plate to form the penile urethra. Ectodermal cells derived from the tip of the glans penetrate inward and form a short epithelial cord. Thus, the penile urethral forms as a result of the fusion of the medial edges of the endodermal urethral folds wherein the fusion of the ectodermal edges of the urethral groove form the median raphe [19].

Hypospadias is a relatively common malformation in the male external urinary tract, typically affecting 4–6 in 1000 live male births [20–23]. The prevalence of hypospadias in international birth defect surveillance systems 1980–2010 is significantly increasing by 0.25 cases/10,000 births/year, although there have been several papers that increases were stabilized of even decreased in recent years [24]. It is characterized by defective development of the penile ventral surface and is defined as a "hypoplasia of the tissues forming the ventral aspect of the penis beyond the division of the corpus spongiosum." Hypospadias is diagnosed by the ventrally deficient prepuce, the proximal meatus and some other abnormal ventral findings, such as downward glans tilt, deviation of the median penile raphe, scrotal encroachment to the penile shaft, midline scrotal cleft, and penoscrotal transposition [25]. Depending on the severity, hypospadias is classified into three categories—mild degree, in which the urethra opens at the anterior portion of the penis (glandular and subcoronal), moderate degree in which the opening is on the middle of the penile

shaft, and severe degree that involves posterior penile, penoscrotal, scrotal, and perineal opening [26].

Hypospadias may affect the psychological, aesthetic, and functional aspects of an individual with complications like urinary dysfunction, sexual dysfunction, and appearance. Chordee and penile hypoplasia, particularly in proximal forms, and abnormal position of the meatus interferes with the normal flux of urine, and penile curvature may cause difficult penetration during sexual intercourse with painful erections [27]. Although more than 200 repairs have been described, hypospadias is corrected by surgery to deal with the following major abnormalities: (1) abnormal ventral curvature or chordee, (2) abnormal proximal insertion, (3) abnormal appearance of the glans penis, (4) abnormal appearance of prepuce [28].

4.4 Lifestyles

There are several pieces of evidence regarding the significance of lifestyle factors, such as diet, smoking, and alcohol consumption as risk factors of cryptorchidism and hypospadias, which were investigated in most of case–control studies. However, these associations remain contentious as following.

4.4.1 Diet

Phytoestrogens have either estrogenic or anti-estrogenic activity depending on the natural environment and their chemical structures. The phytoestrogens with anti-estrogenic effects have recently received extensive coverage, as the experimental findings revealed that increased dietary intakes of phytoestrogens have a protective effect on the risk of various hormone-related diseases, particularly breast cancer [29].

A longitudinal study with 8000 participants in the UK revealed five times increased risk of having a hypospadias-affected son born to women with a vegetarian diet. This could be attributed to greater exposure of vegetarians to phytoestrogens such as soy products, than the omnivores. These results supported the possibility of phytoestrogens having a deleterious effect on the developing hypospadias [30].

However, two independent case–control studies revealed that the vegetarian diet was not associated with the prevalence of hypospadias [31].

Further, in addition to vesitalian diet, a larger case–control study also found a diet during pregnancy lacking both fish and meat was associated with a more than a 4- fold increased risk of hypospadias [odds ratio (OR) = 4.6; 95% confidence interval (CI), 1.6–13.3] [32]. Contrary to this, Giordano et al. reported a positive association between hypospadias and frequent consumption of fish and market purchased fruit. However, the association could be confounded by potential endocrine-disrupting effects from specific components of foods [33].

This field needs to be further clarified by additional carefully performed research.

4.5 Smoking/Alcohol

Several studies have reported the association between smoking and cryptorchidism. Zhang et al. revealed in a systematic review and meta-analysis of studies that maternal gestational smoking was associated with an increased risk of cryptorchidism in their children [34]. Similarly, Kurihashi et al. showed paternal smoking before and during pregnancy as one of the risk factors leading to cryptorchidism in a case–control study [35]. In contrast, no association was found between maternal smoking and the prevalence of hypospadias in other case–control studies [31, 32, 36–38].

Alcohol consumption during pregnancy was found to be associated neither with cryptorchidism [39] nor hypospadias [37] in case–control studies. The findings were further supported by Meyer et al. [38] and Brouwers et al. [31] reporting the same results in other case–control studies.

Thus, the effects of alcohol and smoking were controversial on the prevalence of cryptorchidism and hypospadias.

4.6 Effect of Environmental Chemicals

EEDs are exogenous agents that interfere with the synthesis, secretion, transport, metabolism, binding action, or elimination of natural hormones that are responsible for homeostasis, reproduction, and developmental processes [40, 41]. It has been reported that exposure to endocrine-disrupting chemicals may modulate the prenatal hormonal environment. Endocrine-disrupting chemicals have been shown to induce a broad spectrum toxic effects on the reproductive system and genital development in the prenatal period in humans, particularly as demonstrated by antiandrogen activity in males [3, 4, 42].

Based on the mechanism of cryptorchidism and hypospadias, endocrine-disrupting chemicals could affect the hormonal environment during the prenatal period in humans (Fig. 4.1). In Table 4.1, we have summarized a list of studies showing the effect of the exposure of parents to endocrine-disrupting chemicals for the prevalence of cryptorchidism and hypospadias. It has been shown that the EEDs affect the reproductive system and genital development in the preconception and prenatal period (Table 4.1).

4.6.1 Phthalates

Phthalate diesters (phthalates) have been used as plasticizers for various plastic products, including toys, food containers, furniture, personal care products, medical devices, and housing materials. There are some reports that prenatal exposure to phthalates affects the hormonal environment in infants. It has been

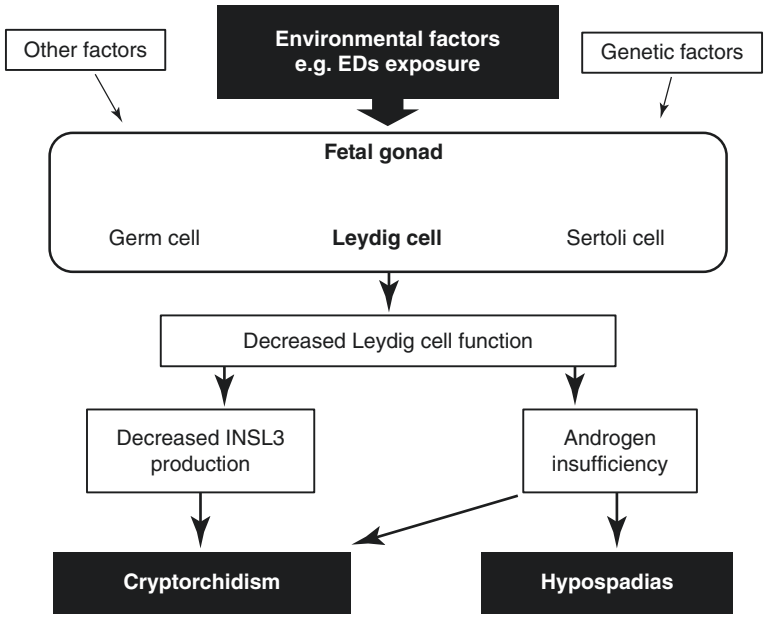


Fig. 4.1 A schematic representation of the influence of EDs on development of cryptorchidism and hypospadias. Exposure to some endocrine-disrupting chemicals in parents may modulate prenatal hormonal environment through fetal gonadal function. Changes in the prenatal hormonal environment may affect the prevalence of cryptorchidism and hypospadias

demonstrated that both DEHP and MEHP can inhibit testosterone production in vitro in human testis explants [43]. Main et al. revealed that the concentrations of phthalate monoesters in breast milk were positively correlated with LH/testosterone ratio and negatively correlated with free testosterone [44]. Jensen et al. revealed a possible association between testosterone and INSL3 levels with phthalate and their metabolites; however, this was not consistently associated with cryptorchidism and hypospadias [45]. Occupational exposure of 74 men to DBP and DEHP increased the concentration of phthalate metabolites MBP and MEHP and was negatively associated with free urinary and serum testosterone levels [46]. Furthermore, an inverse correlation between MEHP and testosterone, estradiol, and free androgen indices was observed [47, 48]. Our cohort study demonstrated that maternal exposure to di(2-ethylhexyl) phthalate in utero might have adverse effects on both Sertoli and Leydig cell development in males and MEHP levels were found to be associated with reduced levels of testosterone, estradiol, inhibin B, and INSL3 in cord blood [49]. In another study, we have also demonstrated the effect of increased maternal MEHP exposures on adrenal androgens (DHEA and androstenedione) and glucocorticoids (cortisol and cortisone) in the cord blood [50]. Conclusively, prenatal exposure to phthalates has the potential to affect the hormonal environment in infants.

Table 4.1 Summary of environmental chemicals for cryptorchidism and hypospadias

Country, study year	Study	Subject	Exposure	Result	Ref.
Nice area, France, 2002–2005	Case–control (95 cryptorchidism, 188 male controls)	Mothers (cryptorchidism)	Anti-rust products Phthalates	6.7% vs. 0.6% controls ($P = 0.005$) 4.8% vs. 0.6% controls ($P = 0.031$)	Wagner-Mahler et al. [51]
United States of America, 1999–2002	Case–control (12 cryptorchidism, 107 male controls)	Mothers (cryptorchidism)	Phthalates (urine) MEHP	Coefficient −1.258 ($P = 0.048$)	Swan et al. [52]
Southeast England, 2000–2003	Case–control (471 hypospadias, 490 male controls)	Mothers (hypospadias)	Phthalates Hair spray Folate	OR = 3.12 (1.04–11.46) OR = 2.39 (1.40–4.17) OR = 0.64 (0.44–0.93)	Ormond et al. [53]
New Zealand	85 children of New Zealand soldiers	New Zealand soldiers (1948–1960)	Dibutyl phthalate (DBP) clothing	Increases in the prevalence Cryptorchidism 5.1% vs. 0.91–1.09% control Hypospadias 2.5% vs. 0.30–0.33% control	Carran and Shaw [54]
Turku, Finland, and Copenhagen, Denmark	Case–control (62 cryptorchid, 68 male controls)	Mothers (cryptorchidism)	Phthalates mEP and mBP mMP, mEP, and mBP miNP mBP	n.s. Positive correlations with SHBG ($r = 0.323$, $p = 0.002$ and $r = 0.272$, $p = 0.01$, respectively) Positively correlated with LH: free testosterone ratio ($r = 0.21$–0.323, $p = 0.002$–0.044) Positively correlated with luteinizing hormone ($r = 0.243$, $p = 0.019$) Negatively correlated with free testosterone ($r = –0.22$, $p = 0.033$)	Main et al. [44]

(continued)

Table 4.1 (continued)

Country, study year	Study	Subject	Exposure	Result	Ref.
England and Wales	Registry-based (2794 hypospadias in 1980–1989 and 677 hypospadias in 1992–1996 from the National Congenital Anomaly System)	Mothers (hypospadias)	Phthalates Hairdressers (the largest group exposed to potential EDCs) (1992–1996)	n.s. OR = 1.50 (1.02–2.09), adjusted for year of birth, region, maternal age	Vrijheid et al. [56]
Western Australia, 1980–2000	Case–control (1202 hypospadias, 2583 male controls)	Mothers (hypospadias) Fathers (hypospadias)	Phthalates Heavy metals Polychlorinated organic compounds Bi-phenolic compounds	OR = 1.2 (0.8–1.7) OR = 2.6 (1.3–5.2) OR = 1.3 (1.0–1.8) OR = 1.6 (1.0–2.6)	Nassar et al. [57]
Denmark, 1980–1996	Case–control (270 cryptorchidism, 75 hypospadias, 300 male controls)	Mothers	Mono(4-methyl-7-carboxyheptyl) phthalate (7cx-MMeHP, DiNP metabolite)	Cryptorchidism OR = 1.28 (0.80–2.01) Hypospadias OR = 1.69 (0.78–3.67)	Jensen et al. [45]
Denmark	Combined with four cohorts (Sons of 646 mothers working in horticulture during pregnancy, 17 cryptorchidism and 11 orchiopexy)	Mothers	Pesticides	Cryptorchidism HR = 1.34 (0.30–5.96) [cohort 2] HR = 2.58 (1.07–6.20) Orchiopexy HR = 1.93 (0.24–15.4) [cohort 4] HR = 2.76 (1.03–7.35)	Gabel et al. [60]
Denmark, 1980–2007	Birth Cohort ($n \geq 600{,}000$)	Mothers (cryptorchidism)	Pesticides Farmers Horticultural workers	HR = 1.31 (1.12–1.53) HR = 1.20 (0.95–1.52)	Jorgensen et al. [61]
Denmark, 1983–1992	Case–control (6177 cryptorchidism, 1345 hypospadias, 23,273 male controls)	Mothers (cryptorchidism)	Pesticides Gardening	OR = 1.67 (1.14–2.47)	Weidner et al. [62]

Denmark, 1983–1992	Case–control (62 cryptorchidism, 68 male controls)	Breast milk	Pesticides Eight most abundant persistent pesticides (*p*,*p*′-DDE, *p*,*p*′-DDT, β-HCH, HCB, α-endosulfan, *cis*-HCE, oxychlordane, and dieldrin)	Combined statistical analysis showed significantly higher in boys with cryptorchidism ($p = 0.032$)	Damgaard et al. [63]
Germany	Case–control (18 cryptorchidism, 30 male controls)	Fat tissue	HCE, HCB	Increases were found in cryptorchidism ($P < 0.05$)	Hosie et al. [64]
Norway, 1967–1991	Registry-based (192,417 births, 251 cryptorchidism, 270 hypospadias)	Parents	Pesticides purchase Pesticides purchase and field vegetables Tractor spraying equipment Tractor spraying equipment and grain	Cryptorchidism OR = 1.70 (1.16–2.50) Cryptorchidism OR = 2.32 (1.34–4.01) Hypospadias OR = 1.38 (0.95–1.99) Hypospadias OR = 1.51 (1.00–2.26)	Kristensen et al. [65]
The province of Ragusa, Sicily, Italy, 1998–2002	Case–control (60 cryptorchidism, 61 hypospadias, 203 male controls)	Mothers Fathers	Employed in agriculture Probable exposure to pesticides Indirect contact with agricultural products (transport and retail)	Cryptorchidism OR = 2.97 (0.77–11.47) Cryptorchidism OR = 2.74 (0.72–10.42) Cryptorchidism OR = 2.45 (0.63–9.59) Hypospadias and cryptorchidism combined OR = 2.24 (0.67–7.48)	Carbone et al. [66]
Eastern Arkansas, United States of America, 1998–2002	Case–control (354 hypospadias, 727 male controls)	Parents (hypospadias)	Diclofop-methyl use Pesticide applications in aggregate Applications of alachlor Permethrin	OR = 1.08 (1.01–1.15) OR = 0.82 (0.70–0.96) OR = 0.56 (0.35–0.89) OR = 0.37 (0.16–0.86)	Meyer et al. [38]

(continued)

Table 4.1 (continued)

Country, study year	Study	Subject	Exposure	Result	Ref.
Washington, United States of America, 1986–1996	Case–control (2395 cryptorchidism, 9580 male controls)	Mothers (cryptorchidism)	Occupation in farming or horticulture Smoking during pregnancy	OR = 1.2 (0.7–2.2) OR = 1.2 (1.1–1.4)	Biggs et al. [67]
The Netherlands 1987–1997	Case–control (583 hypospadias, 251 male controls)	Fathers Mothers (hypospadias)	Pesticides DES	OR = 2.1 (0.6–7.1) OR = 3.5 (0.8–15.6)	Brouwers et al. [31]
The Netherlands, 1996–2004	Case–control (205 hypospadias, 629 boys with middle ear effusion)	Mothers Fathers	Pesticides DES Pesticides	OR = 0.8 (0.2–2.4) OR = 1.6 (0.7–3.4) OR= 0.8 (0.4–1.6)	Brouwers et al. [36]
California, United States of America, 1991–2004	Case–control (690 hypospadias, 2195 male controls)		Pesticides (pesticide applications within a 500-m radius of mother's residential address)	n.s.	Carmichael et al. [68]
United States of America, 1959–1965	Case–control (217 cryptorchidism, 197 hypospadias, 557 male controls)	Mothers	Trans-nonachlor and oxychlordane measured in third-trimester serum	n.s.	Trabert et al. [69]
Korea	Case–control (hypospadias, male controls)	Mothers	DEHP (urine) n-NP (urine) PA (plasma) BPA (plasma)	$P = 0.006$ $P = 7.26 \times 10^{-6}$ $P = 0.009$ $P = 7.22 \times 10^{-10}$	Choi et al. [73]
Granada province, Southern Spain, 2000–2002	Case–control (28 cryptorchidism and/or hypospadias, 51 male controls)	Mothers (placenta)	BPA Propyl-PB	OR = 7.2 (1.5–35.5) for malformations OR = 6.4 (1.2–35.5) for malformations	Fernandez et al. [74]
Nice, France, 2002–2005	Case–control (52 cryptorchidism, 128 male controls)	Mothers (cryptorchidism)	BPA (cord blood) MBP (milk)	Negatively correlated with INSL3 ($P = 0.01$; $R^2 = 0.05$) trend for increased ($P = 0.09$)	Chevalier et al. [75]

Nice, France	Case–control (67 cryptorchidism, 84 male controls [cord blood]; 56 cryptorchidism, 69 male controls [colostrums])	Mothers (cryptorchidism)	ΣPCB (milk) DDE (milk)	Higher individual scores of exposure for PCBs, DDE OR = 2.74 (1.15–6.53), $P < 0.022$ OR = 2.16 (0.94–4.98), $P = 0.071$	Brucker-Davis et al. [80]
Denmark, Finland, 1997–2001	Case–control (95 cryptorchidism, 185 male controls [placenta]; 62 cryptorchidism, 68 male controls [breast milk])	Mothers (cryptorchidism)	PBDEs (milk) sum of BDEs 47, 153, 99, 100, 28, 66, and 154	Significantly higher Median, 4.16 vs. 3.16 ng/g fat; $p < 0.007$ A positive correlation with serum luteinizing hormone ($p < 0.033$)	Main et al. [81]
United States of America, 1959–1965	Case–control (230 cryptorchidism, 201 hypospadias, 593 male controls)	Mothers	The sum of PCBs (third-trimester serum samples)	A trend of association between hypospadias and PCBs level 0–1.9 μg/L, reference group 2–2.9 μg/L, OR = 1.57 (1.05–2.34) 3–3.9 μg/L, OR = 1.45 (0.90–2.34) ≥ 4.0 μg/L, OR = 1.69 (1.06–2.68) *P*-value for trend = 0.08	McGlynn et al. [82]
California, United States of America	Case–control (20 hypospadias, 28 male controls)	Mothers (hypospadias)	PBDEs, PCBs (mid-pregnancy serum samples)	n.s.	Carmichael et al. [83]
South of France	Multi-institutional study (408 hypospadias and 302 male controls)	Mothers	Paints/solvents, detergents, pesticides, cosmetics, other industrial chemicals, herbicides	Fetal exposure to EDCs around the window of genital differentiation was more frequent in the case of hypospadias (40.00% vs. 17.55%), OR = 3.13 (2.11–4.65)	Kalfa et al. [123]

BDEs brominated diphenyl ethers, *BPA* bisphenol A, *DDE* dichloro-diphenyl-trichloro-ethylene, *DEHP* di-(2-ethylhexyl)phthalate, *HCB* hexachlorobenzene, *HCE* heptachlor epoxide, *HCH* hexachlorocyclohexane, *HR* hazard ratio, *MBP* monobutyl phthalate, *NP* nonylphenol, *OR* odds ratio, *PA* phthalic acid, *PB* parabens, *PBDEs* polybrominated diphenyl ethers, *PCBs* polychlorinated biphenyls, *vs.* versus

However, to establish the association between phthalates and prevalence of cryptorchidism and hypospadias, pieces of evidence remain controversial. Wagner-Mahler et al. revealed that the rate of cryptorchidism was higher in children born to mothers with occupational phthalate exposure [51]. A study involving 119 boys of whom both testicular location and phthalate concentrations were available revealed that higher DEHP metabolite concentration was significantly associated with a greater probability of cryptorchidism, wherein no other phthalate metabolites were found to be associated with testicular descent [52].

On the other hand, studies reported that anti-androgenic EDCs might play a role in hypospadias. A case–control study involving 471 hypospadias and 490 randomly selected birth controls, reported an increased risk of hypospadias for phthalate exposure obtained by a job exposure matrix (OR = 3.12; 95% CI, 1.04–11.46) [53]. Carran and Shaw reported that in the children of New Zealand soldiers who served in Malaya (1948–1960) and were exposed to DBP applied daily to their clothing as an acaricide to prevent tick-transmitted bush typhus, the prevalence of hypospadias and cryptorchidism had been increased as compared to the general population [54].

On the contrary, some studies have reported the negative association between phthalates and prevalence of cryptorchidism and hypospadias. Chevrier et al. revealed that there was no significant association between occupational phthalate exposure and prevalence of cryptorchidism and hypospadias [55]. Similarly, in a cohort with 62 cryptorchid and 68 healthy boys, no association was found between phthalate monoester levels and cryptorchidism [44]. Even in hypospadias, Vrijheld et al. reported no association between exposure to phthalates and the prevalence of hypospadias, which was adjusted for social class, year of birth, region, and maternal age [56]. Nassar et al. also showed no association between exposure to phthalates and the prevalence of hypospadias in a large registry-based case–control study in Australia, although women exposed to phthalates were more likely to have an affected hypospadias son (OR 1.2; 95% CI, 0.8–1.7) [57]. In a cohort with 270 cryptorchidism cases, 75 hypospadias cases, and 300 controls, metabolites of DEHP and DiNP ($n = 645$) and steroid hormones ($n = 545$) in amniotic fluid samples were measured, which revealed that none of the metabolites were consistently associated with cryptorchidism or hypospadias. However, from the data of testosterone and INSL3 levels, the authors claimed a possible association of phthalate and their metabolites with cryptorchidism and hypospadias [45].

From the studies reporting a contradictory association between phthalates exposure and cryptorchidism and hypospadias, any satisfactory link cannot be established between them, because very short half-life of phthalates may be implicated in differences in exposure levels between studies.

4.6.2 Organochlorine Pesticides (OCPs)

OCPs, which are chlorinated hydrocarbons, had been previously used for agriculture, and they are still detected in the environment and in human populations even after most OCPs have been prohibited. OCPs possess the endocrine-disrupting

properties for our health, particularly hormonal environment. Warembourg et al. reported that prenatal exposure to OCP affected hormone levels at birth. This study revealed that higher levels of HCB and HCE were associated with reduced levels of testosterone and elevated levels of SHBG among boys. Further, HCE was also shown to be associated with higher levels of estradiol and a lower testosterone–estradiol ratio among girls. However, there was no association between *p*,*p*′-DDE and any hormone levels [58]. Our group also demonstrated that in boys, even in low levels, the OCPs were positively correlated with estradiol–testosterone and adrenal androgen–glucocorticoid, but negatively correlated with androstenedione–DHEA, testosterone–androstenedione, corticoid-cortisone, and the FSH-inhibin B ratio [59]. Thus, prenatal exposure to OCPs has the potential to affect the hormonal environment in infants.

Studies reporting the prevalence of cryptorchidism and hypospadias after occupational exposures to OCPs during prenatal period have shown contradicting results. Gabel et al. conducted a cohort study of pregnant women working in horticulture using four cohorts in nationwide Danish health registers to assess the risk of cryptorchidism among exposed horticulture workers compared to the background population, and unexposed horticulture workers. This study revealed that the prevalence of cryptorchidism was slightly higher in sons born to women employed in horticulture who were exposed to pesticides [60]. In a cohort in Denmark, Jorgensen et al. reported sons of maternal farmers were at increased risk of cryptorchidism (157 cases; HR 1.31, 95% CI 1.12–1.53) compared to boys of mothers in other occupations (15,511 cases). However, maternal occupation as a horticultural worker was not significantly associated with an increased risk of cryptorchidism (72 cases; HR 1.20, 95% CI 0.95–1.52). Further, sons of maternal farmers or horticultural workers who likely worked in the first trimester were not at increased risk of cryptorchidism. Since this study was register-based, it is unresolved whether individual pregnant women with high pesticide exposure might present a substantial risk for cryptorchidism among sons [61]. A register-based case–control study involving 6177 cases of cryptorchidism, 1345 cases of hypospadias, and 23,273 controls revealed a significantly increased risk of cryptorchidism but not hypospadias in sons of women working in gardening (adjusted OR = 1.67; 95% CI, 1.14–2.47). The risks were not increased in sons of men working in farming or gardening. The increased risk of cryptorchidism among sons of female gardeners could suggest an association with prenatal exposure to occupationally related chemicals [62]. In a prospective birth cohort of case–control study with 62 milk samples from mothers of cryptorchid boys and 68 from mothers of healthy boys, Damgaard et al. revealed the association between cryptorchidism and some persistent pesticides in breast milk. The pesticides included *p*,*p*′-DDT, *p*,*p*′-DDE, *p*,*p*′-DDD, *o*,*p*′-DDT, HCH (α, β, γ), HCB, PCA, α-endosulfan, *cis*-HE, chlordane (*cis*-, *trans*-) oxychlordane, methoxychlor, OCS, and dieldrin [63]. Increased levels of heptachlor epoxide (HCE) and hexachlorobenzene (HCB) were detected in the fat tissue of the patients with cryptorchidism [64]. Regarding hypospadias, paternal occupational exposures to polychlorinated organic (OR 1.3; 95% CI, 1.0–1.8) and bi-phenolic (OR 1.6; 95% CI, 1.0–2.6) compounds were posed as possible risk factors [57]. In congenital disabilities (N = 4565) reported to the Medical Birth Registry of Norway among

192,417 births between 1967 and 1991 to parents identified as farmers in five agricultural and horticultural censuses between 1969 and 1989, there was an association with pesticides for cryptorchidism and hypospadias [65].

On the other hand, several reports showed no association between OCPs and prevalence of cryptorchidism and hypospadias. Carbone et al. reported that an increasing rate of cryptorchidism and hypospadias with increasing pesticide exposure in a Sicilian study; however, there was no significant difference [66]. Meyer et al. did not observe an increase in the risk of hypospadias on estimated exposure to pesticides known to have reproductive, developmental, or endocrine-disrupting effects [38]. Biggs et al. revealed that working in farming or horticulture place was not a risk factor of cryptorchidism [67]. It was also shown that paternal exposure to pesticides before pregnancy does not seem to be associated with hypospadias [31, 36]. A case–control study with cases (n = 690) ascertained by the California Birth Defects Monitoring Program and controls selected randomly from the birth population (n = 2195) investigated the associations of hypospadias prevalence with exposures to physicochemical groups of pesticides and specific chemicals. The study revealed that there was no association between the risk of hypospadias and most of the pesticides used. However, the authors insisted that the results should be interpreted with caution until replicated in other study populations [68]. In a study under the Collaborative Perinatal Project (CPP), pregnant women at 12 US medical centers from 1959 to 1965, consisting of the mothers of 217 sons with cryptorchidism, 197 sons with hypospadias, and 557 sons with neither condition, were enrolled to investigate the effect of chlordane exposure in utero. This study revealed no association between chlordane levels and cryptorchidism or hypospadias [69].

Thus, the association between OCPs and prevalence of cryptorchidism and hypospadias is still unclear, because this may be implicated in designs of study, such as register-based studies comparing workers and gardeners to others.

4.6.3 Bisphenol A (BPA)

BPA is widely used in plastics such as food and drink containers and as an additive in thermal paper, dental sealant, medical equipment, and flame retardant. BPA has a weak estrogenic property as an endocrine disruptor, and prenatal exposure has the potential to affect fetal health.

It has been reported that BPA binds to ERα and ERβ inducing alterations at several levels of organization, including tissues, cells, and gene expression [70]. Further, in cross-sectional analyses conducted in the Chianti population, exposure to BPA induced changes in total testosterone concentrations in men [71]. Our study also revealed that BPA affected prenatal hormone environment. BPA level had a non-significant positive association with testosterone, estradiol, and progesterone levels and a weak negative association with Prolactin (PRL) in boys. In girls, there was a weak positive association between BPA and PRL [72].

Several controversial reports are available explaining the association between phthalates and prevalence of cryptorchidism and hypospadias. In a study conducted in Korean newborn boys with hypospadias, the urine and plasma levels of BPA and phthalic acid were higher compared to controls [73]. Further, in a cohort of newborns recruited between 2000 and 2002 with 28 cases of hypospadias or cryptorchidism and 51 healthy controls nested, the concentration of BPA in placenta tissue was identified as a risk factor for the male genital development [74].

However, Chevalier et al. reported a cohort with 52 cryptorchidism (26 transient, 26 persistent) and 128 control boys born at two hospitals in southern France, who were systematically screened at birth for cryptorchidism over a period of 3 years (2002–2005), and a diagnosis of cryptorchidism confirmed by a senior pediatrician. This study revealed that the concentration of BPA was not significantly different between control and cryptorchidism, although BPA levels of cord blood were negatively correlated with INSL3 [75].

Thus, there are not adequate data to establish an association between exposure to BPA and cryptorchidism or hypospadias. Very short half-life of BPA may be also implicated in differences in exposure levels between studies should be also considered in investigation.

4.6.4 Dioxins/Dioxin-Like Compounds

In addition to dioxins, 17 PCDDs/PCDFs and 12 PCBs have been categorized as dioxin-like compounds (DLCs) in the World Health Organization-International Programme on Chemical Safety expert meeting, 2005 [76]. DLCs disrupt normal fetal development through binding to Aromatic hydrocarbon receptors (AHRs), which regulate xenobiotic metabolism, cell proliferation, and cell cycle control in fetal tissues [76].

In Yucheng, over 2000 Taiwanese people ingested rice oil accidentally contaminated with PCBs and PCDFs between 1978 and 1979. Serum hormone levels, in addition to physical examination, were investigated in 61 control boys and 60 Yucheng boys. Yucheng boys were born to women accidentally exposed to high levels of DLCs after consuming DLC-contaminated rice oil. The study showed increased levels of serum estradiol and serum follicle-stimulating hormone levels and decreased serum testosterone levels in Yucheng boys at the age of puberty [77]. In a birth-cohort study in the industrialized city of Duisburg, Germany, Cao et al. revealed that fT and E2 concentrations in the cord blood were inversely correlated with maternal PCDD/PCDF concentrations [78]. Our study also investigated in cord blood samples showed that maternal DLCs was negatively correlated with T/E2 ratios and SHBG levels and positively correlated with DHEA levels and AA (sum of DHEA and androstenedione)/GC (sum of cortisol and cortisone) ratios in boys [79]. Thus, dioxins and DLCs affect the environment of reproductive and steroid hormones during the prenatal period.

There are only a few human-based reports available to unravel the association between dioxins/DLCs and prevalence of cryptorchidism and hypospadias. In a

prospective case–control study consisting of 151 cord blood samples (67 cryptorchidism, 84 tightly matched controls) and 125 colostrums (56 for cryptorchidism and 69 for controls) were screened for xenobiotics, including anti-androgenic polychlorinated biphenyls (PCBs). This study supported an association between congenital cryptorchidism and fetal exposure to PCBs [80]. In a prospective Danish–Finnish study with individual breast milk samples of 62 cryptorchidism and 68 controls for 14 polybrominated diphenyl ethers (PBDEs), the concentration of PBDEs in breast milk was found to be significantly higher in boys with cryptorchidism than in controls [81].

However, no association between maternal serum PCBs levels and cryptorchidism or hypospadias was found in a large, well described, population in the USA [82]. Hosie et al. also revealed in a small cohort that PBBs in the fat sample was not associated with the prevalence of cryptorchidism [64]. In a cohort with 40 hypospadias and 18 controls, Carmichael et al. revealed that levels of the PBDEs and PCBs were not statistically significantly different between groups [83].

Thus, exposure to dioxins/DLCs during the prenatal period and its association with the prevalence of cryptorchidism and hypospadias is not clear.

4.6.5 PFOS/PFOA

Of the Perfluoroalkyl substances (PFASs), which are widely used in industrial products, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are the most commonly detected PFASs in the environment and humans. PFOS was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants in 2009 and PFOA has been proposed to be listed on Stockholm Convention by EU.

In a cross-sectional study involving children of 6–9 years old in Mid-Ohio Valley, PFOA level was found to be significantly associated with testosterone whereas PFOS were significantly associated with estradiol and testosterone in boys [84]. In a pregnancy cohort established in Aarhus, Denmark, in utero exposure to PFOA, not PFOS was associated with higher adjusted levels of luteinizing hormone and follicle-stimulating hormone [85]. In our study, maternal exposure to perfluorinated chemicals also affected the prenatal environment of sex hormones as well as adrenal hormones [86, 87].

Thus, prenatal exposure to PFASs disrupts reproductive functions by altering reproductive hormone secretions at a later age. However, Vesterholm Jensen et al. reported that no statistically significant association between PFOA and PFOS levels in cord blood and cryptorchidism were found in case–control study among 215 boys from Denmark and Finland [88]. Further, there was no significant data in humans to establish the association between prenatal exposure to PFASs and prevalence of hypospadias. Hence, attempts should be made to investigate the effect of prenatal exposure to PFASs on the prevalence of cryptorchidism and hypospadias.

4.7 Genes and Polymorphisms

Several studies revealed that genetic susceptibility to the effect of EEDs might affect the development of cryptorchidism and hypospadias. This is known as gene–environment interaction. There are several reports available showing the effect of genes and polymorphism on cryptorchidism and hypospadias. We have summarized some of those studies in Table 4.2.

Aschim et al. investigated polymorphic CAG and GGN segments, which regulate androgen receptor (AR) function, in patients diagnosed with hypospadias ($n = 51$), cryptorchidism ($n = 23$), and controls ($n = 210$). The study revealed that GGN length was associated with the risk of cryptorchidism and penile hypospadias; meanwhile, there were no significant differences in CAG lengths in hypospadias and cryptorchidism compared to control boys [89]. Radpour et al. also revealed the same results in a case–control study of 76 cryptorchidism and 92 hypospadias. This study identified 12 different CAG alleles and 8 different GGN alleles in the cryptorchid group [90]. Other studies also supported these results [91, 92]. On the other hand, Wiener et al. reported that mutations of the androgen receptor gene in the hormone and DNA binding domains of the protein were not associated with cryptorchidism [93].

On the other hand, the genetic polymorphism of ESR1 was reported not to be associated with cryptorchidism [94, 95]. However, Beleza-Meireles et al. investigated mutations in the coding regions of ESR1 and ESR2 genes and genotyped the CA repeat polymorphism in 60 boys with hypospadias. The CA repeat polymorphism in ESR2 was observed to be prolonged in hypospadias patients compared to controls, which was associated with lower levels of testosterone. The genetic variants of rs944050 and rs3832949 in ESR2 were also more in hypospadias patients as compared to controls. The findings of this study revealed that abnormalities of ESR2, not ESR1, are involved in the prevalence of hypospadias [96].

In contrast, few reports have shown the involvement of ESR1 in hypospadias. Choudhry et al. reported that ESR1 single nucleotide polymorphisms and haplotypes influence the risk of hypospadias in White and Hispanic subjects [97]. Ban et al. reported that genetic polymorphisms of both ESR1 and ESR2 were associated with the incidence of hypospadias [98]. Watanabe et al. revealed that homozygosity for the specific ESR1 “AGATA” haplotype was a risk factor of hypospadias [99].

There are some other genes associated with cryptorchidism and hypospadias. The genetic polymorphism of INSL3 has been reported as one of the risk factors for cryptorchidism [100]. However, some studies also have reported that there was no association between the genetic variant and cryptorchidism [101, 102]. In addition, pieces of evidence are available reporting the association of Hox genes, with cryptorchidism. Kolon et al. reported genetic polymorphism of the HOXA10 gene in cryptorchidism. However, this polymorphism was also identified in normal boys [103]. Lu et al. revealed that polymorphisms in the HOXA11 gene contributed to the risk of developing unilateral cryptorchidism [104]. Wang et al. reported that the variant 180A>G (A60A) in HOXD13 is also a risk factor for cryptorchidism [105]. Among other genes, Ars et al. reported a more frequent appearance of the T22P variant of RXFP2 with a mild risk of cryptorchidism in the Italian population than

Table 4.2 Summary of genes and polymorphisms involved in cryptorchidism and hypospadias

Design	genes	SNPs	Subject	Genotype	Outcome	Ref.
Case–control (92 hypospadias, 76 cryptorchidism, and 190 controls)	AR	GGN repeats	Penile hypospadias Cryptorchidism	GGN number (mean ± SD)	23.9 ± 1.9 ($P = 0.018$) 24.5 ± 2.4 ($P = 0.001$)	Radpour et al. [90]
Case–control (105 cryptorchidism and 115 controls)	AR	CAG and GGC repeats	Bilateral cryptorchidism	CAG = 21/GGC = 18 CAG ≥ 21/GGC ≥ 18	16.0% vs. 5.2% in controls ($P < 0.05$) 38.0% vs. 20.9% in controls ($P < 0.05$)	Ferlin et al. [91]
Case–control (51 hypospadias, 23 cryptorchidism, and 210 controls)	AR	GGN repeats	Penile hypospadias Cryptorchidism	GGN number [median (range)]	24 (23–26) ($P = 0.003$) 24 (23–24) ($P = 0.001$)	Aschim et al. [89]
A meta-analysis of five studies (294 cryptorchidism and 646 controls)	AR	CAG and GGN repeats	Bilateral cryptorchidism Cryptorchidism	CAG repeat length GGN repeat length [WMD (95% CI)]	0.74 (0.01–1.47) ($P < 0.05$) 1.17 (0.28–2.06) ($P < 0.05$)	Wang et al. [92]
Case–control (21 cryptorchidism)	AR	Exons 2–8	Cryptorchidism		n.s.	Wiener et al. [93]
Case–control (48 cryptorchidism, 20 controls)	AR	Exons 1–8	Cryptorchidism		n.s.	Suzuki et al. [124]
Case–control (1113 cryptorchidism, 740 controls)	ESR1	TA repeats	Cryptorchidism	TA repeats	n.s.	Lo Giacco et al. [94]
Case–control (70 micropenis, 43 hypospadias, 80 spermatogenic failure and 135 controls)	ESR1	"AGATA" haplotype	Hypospadias Micropenis	"AGATA" haplotype	OR =11.26 ($P = 0.0000033$) OR = 3.64 ($P = 0.040$)	Watanabe et al. [99]

Case–control (152 cryptorchidism, 160 controls)	ESR1	rs6932902	Severe cryptorchidism	GG genotype G allele	93% vs. 54% moderate ($P = 0.04$) 97 % vs. 76% moderate ($P = 0.03$)	Wang et al. [95]
Case–control (60 hypospadias, 152 cryptorchidism, 160 controls)	ESR1 ESR2	CA repeats rs944050 (2681-4A>G)	Hypospadias	CA repeats	Prolonged ($P < 0.05$) The heterozygous form is more frequent ($P < 0.05$)	Beleza-Meireles et al. [96]
Case–control (59 hypospadias, 286 controls)	ESR1 ESR2	rs2234693 (PvuII) rs9340799 (XbaI) rs944050 (2681-4A>G)	Hypospadias	AG/GG vs. AA CG haplotype AG/GG vs. AA	n.s. OR = 0.52 ($P < 0.05$) $X^2 = 5.088$ ($P < 0.05$) OR = 0.59 ($P < 0.05$)	Ban et al. [98]
Case–control (647 hypospadias, 877 controls)	ESR1 ESR2	33 of 108 SNPs 6 haplotype blocks 36 SNPs	Hypospadias		OR >1, $P = 0.05$–0.007 OR = 1.3–1.8, $p = 0.04$–0.00001 n.s	Choudhry et al. [97]
Case–control (158 hypospadias, 90 control)	SRD5A2	rs523349 (V89L)	Hypospadias	Homozygous V allele	OR = 0.24 (0.14–0.41)	Thai et al. [125]
Case–control (89 hypospadias, 291 control)	SRD5A2 HSD17B3	rs523349 (V89L) rs2066479 (G289S)	Hypospadias Severe hypospadias Hypospadias Severe hypospadias	CC/CG vs. GG CC/CG vs. GG AA vs. AG/GG AA vs. AG/GG	n.s. OR = 3.19 (1.09–9.36) OR = 3.06 (1.38–6.76) OR = 3.93 (1.34–11.49)	Sata et al. [126]
Meta-analysis (1130 hypospadias, 1279 controls)	SRD5A2	rs523349 (V89L)	Hypospadias	C vs G Codominant model: CC vs GG GC vs GG Dominant model (GC + CC vs GG) Recessive model (CC vs GC + GG)	OR 1.91 (1.13–3.23), $P = 0.02$ OR 2.97 (1.25–7.04), $P = 0.01$ OR 2.36 (1.35–4.13), $P = 0.003$ OR 2.46 (1.28–4.72), $P = 0.007$ OR 1.91 (1.00–3.66), $P = 0.05$	Zhang et al. [109]

(continued)

Table 4.2 (continued)

Design	genes	SNPs	Subject	Genotype	Outcome	Ref.
Case–control (31 hypospadias, 64 controls)	CYP1A1 GSTM1 GSTT1	rs4646903 (MspI) null null	Hypospadias	CC/CT vs. TT	OR = 0.28 (0.08–0.97), $P = 0.044$ n.s. n.s.	Kurahashi et al. [119]
Case–control (95 cryptorchidism, 98 hypospadias, 141 controls in Japanese; 58 cryptorchidism, 129 controls in Italian)	ARNT2 CYP1A2 CYP1A1 NR1I2 AHR ARNT2 NR1I2-CYP1A1-ARNT2 CYP1A2-ARNT2 ARNT2-AHR	rs2278705, rs5000770 rs2069521 rs4919686 rs2472680 rs3757824 rs1020397 Synergistic interaction between rs2472680, rs4919686 and rs5000770 Synergistic interaction between rs2069521 and rs2278705 Multi-locus association between rs5000770 and rs3757824	Japanese C/H Italian cryptorchidism Japanese cryptorchidism Japanese hypospadias Combined Japanese and Italian C		Allele frequency ($P < 0.05$) Allele frequency ($P < 0.05$) 62.81% prediction accuracy ($P = 0.011$) 69.98% prediction accuracy ($P = 0.001$) 65.70% prediction accuracy ($P = 0.055$)	Qin et al. [121]
Preputial tissues Case–control (23 hypospadias, 16 controls (phimosis))	CYP1A1 CYP1B1 AR SRD5A2		Hypospadias	mRNA expression	n.s. Higher ($P < 0.001$) Lower ($P < 0.001$) n.s. Negative correlations between the methylation level of SRD5A2 and the CYP1 family mRNA expression levels (CYP1A1, $p = 0.002$; CYP1B1, $p = 0.007$)	Ohsako et al. [118]

Case–control (85 cryptorchidism, 99 controls)	INSL3 RXFP2 HOXA10	rs121912556 p.R105R rs10421916 rs1555633 rs7325513 rs3779456	Cryptorchidism	A heterozygous form A heterozygous form	None in controls None in controls Allele frequency ($P < 0.05$) Allele frequency ($P < 0.05$) Allele frequency ($P < 0.05$) Allele frequency ($P < 0.05$)	Chavez-Saldana et al. [100]
Case (5 cryptorchidism)	INSL3	Ala/Thr60	Cryptorchidism		n.s.	Takahashi et al. [101]
Case–control (118 cryptorchidism, 18 controls)	INSL3	G/A	Cryptorchidism		n.s.	Baker et al. [102]
Case–control (45 cryptorchidism, 16 control)	HOXA 10	Exons 1 and 2	Cryptorchidism		n.s.	Kolon et al. [103]
Case–control (168 cryptorchidism, 193 controls)	HOXA11	rs6461992	Cryptorchidism		n.s.	Lu et al. [104]
Case–control (44 cryptorchidism, 46 controls)	HOXA13	180A>G (A60A)	Cryptorchidism		Allele frequency ($P < 0.05$)	Wang et al. [105]
Case–control (187 cryptorchidism, 390 controls [Spain]; 199 cryptorchidism, 351 controls [Italia])	RXFP2	T222P	Cryptorchidism	T222P	Allele frequency ($P < 0.05$)	Ars et al. [106]
Case–control (113 cryptorchidism, 179 controls)	AXIN1	rs370681 rs1805105	Cryptorchidism		Allele frequency ($P < 0.05$) Allele frequency ($P < 0.05$)	Zhou et al. [107]
Human foreskin fibroblast cells (hFFCs) Case–control (8 cryptorchidism, 11 hypospadias, 5 controls)	MMP11 NTSR1 ARNT2	rs5000770	Hypospadias	Expression Expression AA vs. AG/GG	BPA exposure inhibit (0.74-fold, $P = 0.0034$) Low-dose BPA induced a significant difference ($P = 0.031$)	Qin et al. [121]

(continued)

Table 4.2 (continued)

Design	genes	SNPs	Subject	Genotype	Outcome	Ref.
Case–control (189 hypospadias, 390 controls)	DGKK	Risk-associated DGKK haplotype	Hypospadias		n.s.	Carmichael et al. [122]
Case–control (466 hypospadias, 402 controls; 1966 hypospadias, 2492 controls)	DGKK	rs1934179, rs4143304, rs9969978, rs1934188, rs4826632, rs4599945 rs1934179, rs4143304, rs9969978, rs1934188, rs7063116, rs1934190 rs1934179	Hypospadias Mild/moderate Hypospadias Severe hypospadias		n.s. ORs >1, $P < 0.05$ (combined) OR >1, $P < 0.05$ (combined)	Ma et al. [108]
Case–control (169 hypospadias, 1148 controls)	HAAO IRX6	rs3816183 rs6499755	Hypospadias Anterior/ middle hypospadias		$P = 0.0019$ $P = 0.0472$	Kojima et al. [111]
Case–control (41 hypospadias, 30 controls)	ATF3	L23M, C53070T, C53632A, Ins53943A	Hypospadias		Genomic variants of ATF3 are present in 10% of our patients with hypospadias (none in controls)	Kalfa et al. [112]
Case–control (330 hypospadias, 380 controls)	ATF3	rs3125289 rs1877474 rs11119982	Hypospadias	TT vs. CC/CT TT vs. CC/CT CC vs. CT/TT	OR = 1.62 (1.13–2.33), $P = 0.02831$ OR = 1.36 (1.01–1.85), $P = 0.01108$ OR = 1.73 (1.19–2.51), $P = 4 \times 10^{-4}$	Beleza-Meireles et al. [113]
Case–control (41 hypospadias, 30 controls)	MAMLD1 (CXorf6)	One missense mutation (1295T>C, V432A) and two deletions (325delG, predicted to cause a stop codon L121X) CAG-repeat	Severe hypospadias Subcoronal hypospadias		None in controls	Kalfa et al. [117]

Case–control (150 hypospadias, 150 controls)	MAMLD1 (CXorf6)	rs41313406 rs2073043	Hypospadias	p.P286S p.N589S SS haplotype	11.3% vs. 8.0%, $p = 0.32$ 14.6% vs. 8.0%, $p = 0.068$ 10.6% vs. 4.0%, $p = 0.044$, OR = 2.87 (1.09–7.55)	Kalfa et al. [127]
Case–control (99 hypospadias, 95 controls)	MAMLD1 (CXorf6)	rs41313406 rs61740566 rs2073043 c.1591ins$(CAG)_3$ c.1585C > A	Hypospadias	p.P286S p.V432A p.N589S p.531ins3Q p.Q529K	11/99 vs. 0/95 controls 1/99 vs. 0/95 controls 11/99 vs. 0/95 controls 3/99 vs. 1/95 controls 1/99 vs. 0/95 controls	Chen et al. [116]

C cryptorchidism, *CI* confidence interval, *H* hypospadias, *n.s.* not significant, *OR* odds ratio, *WMD* weighted mean difference, *vs.* versus

the Spanish population [106]. Zhou et al. revealed that polymorphisms of AXIN1 were associated with cryptorchidism [107]. The role of genes in cryptorchidism is still controversial, and further studies are necessary.

In hypospadias, there are several reports regarding genes and polymorphisms. Ma et al. reported that DGKK is a common risk factor for hypospadias in Caucasian populations, but not in the Chinese Han population [108]. Zhang et al. revealed that the SRD5A2 gene V89L polymorphism is associated with an increased risk of hypospadias, and the C allele was a genetic risk factor of hypospadias [109]. Samtani et al. also reported that V89L polymorphism of SRD5A2 was associated with hypospadias [110]. Kojima et al. investigated candidate genes in hypospadias using a genome-wide study, and showed that the genetic variations of HAAO and IRX6 were associated with hypospadias in the Japanese population [111]. Kalfa et al. [112] and Beleza-Meireles et al. [113] reported that the ATF3 gene variants were associated with hypospadias. The involvement of MAMLD1 (CXor6) has also been reported to be associated with hypospadias [114]. Ogata et al. revealed that the MAMLD1 mutations caused hypospadias though testosterone production during the critical period for sex development [115]. The studies of Chen et al. [116] and Kalfa et al. [117] provided additional support for the association of MAMLD1 with hypospadias.

In order to elucidate the association of chemical exposure with the expression of target regulatory genes involved in male reproductive organ malformations, Ohsako et al. investigated the atypical CYP1 family genes as potential biomarkers of environmental chemical exposure along with SRD5A2 and AR genes in preputial tissues from patients with hypospadias ($n = 23$) and phimosis ($n = 16$). Differences in expression levels of CYP1B1, SRD5A2, and AR genes were found in hypospadias patients compared to the phimosis group. Methylation in the SRD5A2 promoter region was also found in hypospadias patients, while no methylation was detected in CYP1A1, CYP1B1, or AR. Negative correlations were found between the methylation level of SRD5A2, especially at the—221 Sp1 site, and the CYP1 family mRNA expression levels in hypospadias patients, but not in phimosis patients. The findings indicated the involvement of chemical exposure in the onset of hypospadias and speculated a possible association of the epigenomic alterations of the SRD5A2 gene with the activation of AHR induced by exogenous environmental chemicals [118]. In our previous case–control study involving 31 case mothers who had boys with hypospadias and 64 control mothers investigated the association of CYP1A1 (Mspl), polymorphism, the gene involved in the metabolization of environmental chemicals and estrogens with hypospadias. This study observed that the heterozygous CYP1A1 and heterozygous and homozygous CYP1A1 were less frequent in the mothers of hypospadias boys than in the control mothers suggesting the mothers with the CYP1A1 MspI variant allele may be associated with a decreased hypospadias risk [119].

To elucidate the effect of genetic variability among individuals on susceptibility to the effects of BPA exposure as a potential cause of hypospadias, a study has been carried out in human foreskin fibroblast cells of cryptorchidism ($n = 8$) and hypospadias ($n = 21$) and male without genital malformations ($n = 5$). BPA exposure was found to inhibit matrix metalloproteinase-11 expression in the hypospadias group only. The single-nucleotide polymorphism rs5000770 (G>A) located within the aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) locus

in the presence of low-dose BPA induced a significant difference in neurotensin receptor 1 (NTSR1) expression between AA and AG/GG groups [120]. In another study, involving 153 cryptorchidism and 98 hypospadias, and 270 controls of Japanese and Italian males, the association between the SNPs of genes involved in environmental endocrine disruptors metabolism on cryptorchidism and hypospadias has been elucidated. The study evaluated the SNPs involved in dioxin binding (AHR and ARNT2), dioxin induction (CYP1A2), estrogen synthesis (CYP17A1), and bisphenol A induction (NR1I2), and concluded that genetic polymorphisms in genes involved in EED metabolism were associated with the development of cryptorchidism and hypospadias [121].

On the other hand, controversial pieces of evidence have also been reported for the association. In a cohort study of 189 hypospadias and 390 male controls born from 1991 to 2003 in California's San Joaquin Valley, the joint effect of pesticide exposure and variants in DGKK genes involved in sex steroid synthesis/metabolism and genes involved in genital tubercle development has been estimated. The study revealed elevated risks associated with joint exposures to selected pesticides and genetic variants. However, since there was no statistical evidence for interaction, interpretation of data should be considered with caution [122].

Thus, genes and polymorphisms involved in EEDs metabolism might influence the risk of male genital malformations (Fig. 4.2).

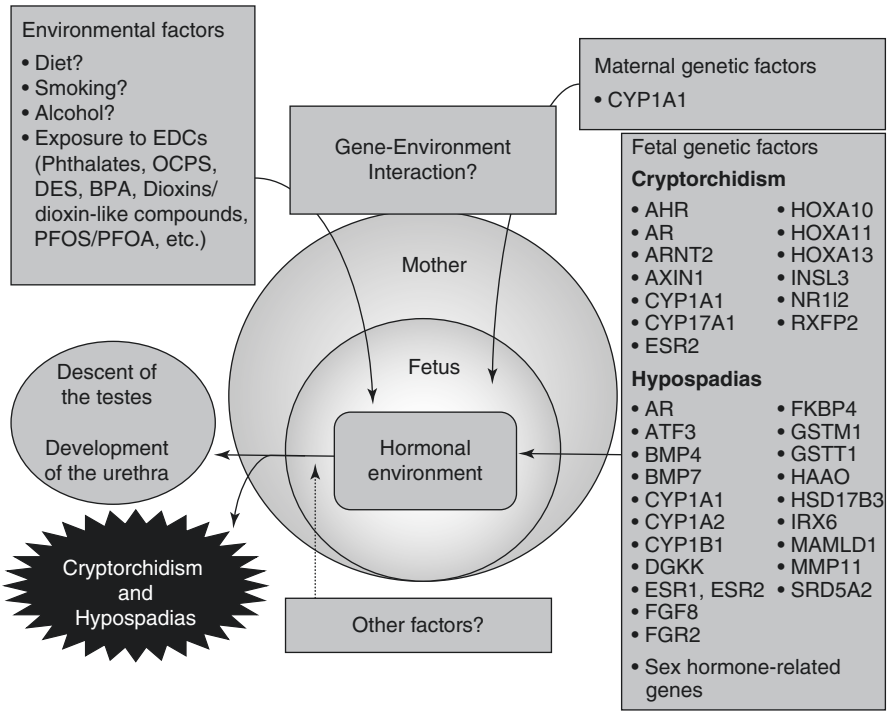


Fig. 4.2 Interaction between environmental and genetic factors for cryptorchidism and hypospadias

4.8 Limitations

Since many factors in addition to environmental chemicals could be associated with incidence of cryptorchidism and hypospadias, it is very difficult to reveal real association from current evidences. Lager studies may be necessary to establish it. Otherwise, compensatory mechanism for exposure to environmental chemicals in our body may mask it. Hence, no association might be "real answer."

4.9 Conclusions

There are not adequate data to establish an association between exposure to environmental chemicals and cryptorchidism or hypospadias. On the other hand, genes and polymorphisms involved in EEDs metabolism might influence the risk of male genital malformations. Thus, to establish a precise association between these factors, further cohort studies are required.

References

1. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001;16(5):972–8.
2. Wohlfahrt-Veje C, Main KM, Skakkebaek NE. Testicular dysgenesis syndrome: foetal origin of adult reproductive problems. Clin Endocrinol. 2009;71(4):459–65.
3. Shih EM, Graham JM Jr. Review of genetic and environmental factors leading to hypospadias. Eur J Med Genet. 2014;57(8):453–63.
4. van der Zanden LF, et al. Aetiology of hypospadias: a systematic review of genes and environment. Hum Reprod Update. 2012;18(3):260–83.
5. Sijstermans K, et al. The frequency of undescended testis from birth to adulthood: a review. Int J Androl. 2008;31(1):1–11.
6. Lane C, et al. A population-based study of prevalence trends and geospatial analysis of hypospadias and cryptorchidism compared with non-endocrine mediated congenital anomalies. J Pediatr Urol. 2017;13(3):284.e1–7.
7. Abdullah NA, et al. Birth prevalence of cryptorchidism and hypospadias in northern England, 1993-2000. Arch Dis Child. 2007;92(7):576–9.
8. Ritzen EM, et al. Nordic consensus on treatment of undescended testes. Acta Paediatr. 2007;96(5):638–43.
9. Trsinar B, Muravec UR. Fertility potential after unilateral and bilateral orchidopexy for cryptorchidism. World J Urol. 2009;27(4):513–9.
10. Hutson JM. A biphasic model for the hormonal control of testicular descent. Lancet. 1985;2(8452):419–21.
11. Hutson JM, et al. Regulation of testicular descent. Pediatr Surg Int. 2015;31(4):317–25.
12. Favorito LA, et al. The importance of the gubernaculum in testicular migration during the human fetal period. Int Braz J Urol. 2014;40(6):722–9.
13. Levard G, Laberge JM. The fate of undescended testes in patients with gastroschisis. Eur J Pediatr Surg. 1997;7(3):163–5.

14. Husmann DA, Levy JB. Current concepts in the pathophysiology of testicular undescent. Urology. 1995;46(2):267–76.
15. Shenker NS, et al. A new role for androgen in testicular descent: permitting gubernacular cell proliferation in response to the neuropeptide, calcitonin gene-related peptide. J Pediatr Surg. 2006;41(2):407–12.
16. Kurzrock EA, Baskin LS, Cunha GR. Ontogeny of the male urethra: theory of endodermal differentiation. Differentiation. 1999;64(2):115–22.
17. van der Werff JF, et al. Normal development of the male anterior urethra. Teratology. 2000;61(3):172–83.
18. Seifert AW, Harfe BD, Cohn MJ. Cell lineage analysis demonstrates an endodermal origin of the distal urethra and perineum. Dev Biol. 2008;318(1):143–52.
19. Baskin LS. Hypospadias and urethral development. J Urol. 2000;163(3):951–6.
20. Sagodi L, et al. Prevalence and possible causes of hypospadias. Orv Hetil. 2014;155(25):978–85.
21. Paulozzi LJ, Erickson JD, Jackson RJ. Hypospadias trends in two US surveillance systems. Pediatrics. 1997;100(5):831–4.
22. Paulozzi LJ. International trends in rates of hypospadias and cryptorchidism. Environ Health Perspect. 1999;107(4):297–302.
23. Dolk H, et al. Toward the effective surveillance of hypospadias. Environ Health Perspect. 2004;112(3):398–402.
24. Yu X, et al. Hypospadias prevalence and trends in international birth defect surveillance systems, 1980-2010. Eur Urol. 2019;76:482–90.
25. Wein AJ, et al. Hypospadias. Campbell-Walsh urology, vol. 4. 11th ed. Philadelphia: Elsevier; 2015. p. 3503–36.
26. Manson JM, Carr MC. Molecular epidemiology of hypospadias: review of genetic and environmental risk factors. Birth Defects Res A Clin Mol Teratol. 2003;67(10):825–36.
27. Manzoni G, et al. Hypospadias surgery: when, what and by whom? BJU Int. 2004;94(8):1188–95.
28. Haudid AT, Azmy AF. Hypospadias surgery. Berlin: Springer; 2004.
29. Bingham SA, et al. Phyto-oestrogens: where are we now? Br J Nutr. 1998;79(5):393–406.
30. North K, Golding J. A maternal vegetarian diet in pregnancy is associated with hypospadias. The ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. BJU Int. 2000;85(1):107–13.
31. Brouwers MM, et al. Risk factors for hypospadias. Eur J Pediatr. 2007;166(7):671–8.
32. Akre O, et al. Maternal and gestational risk factors for hypospadias. Environ Health Perspect. 2008;116(8):1071–6.
33. Giordano F, et al. Maternal diet and the risk of hypospadias and cryptorchidism in the offspring. Paediatr Perinat Epidemiol. 2008;22(3):249–60.
34. Zhang L, et al. Maternal gestational smoking, diabetes, alcohol drinking, pre-pregnancy obesity and the risk of cryptorchidism: a systematic review and meta-analysis of observational studies. PLoS One. 2015;10(3):e0119006.
35. Kurahashi N, et al. Parental and neonatal risk factors for cryptorchidism. Med Sci Monit. 2005;11(6):CR274–83.
36. Brouwers MM, et al. Hypospadias: risk factor patterns and different phenotypes. BJU Int. 2010;105(2):254–62.
37. Hussain N, et al. Hypospadias and early gestation growth restriction in infants. Pediatrics. 2002;109(3):473–8.
38. Meyer KJ, et al. Agricultural pesticide use and hypospadias in eastern Arkansas. Environ Health Perspect. 2006;114(10):1589–95.
39. Strandberg-Larsen K, et al. Alcohol binge drinking during pregnancy and cryptorchidism. Hum Reprod. 2009;24(12):3211–9.
40. Kavlock RJ, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. Environ Health Perspect. 1996;104(Suppl 4):715–40.

41. Diamanti-Kandarakis E, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev. 2009;30(4):293–342.
42. Lymperi S, Giwercman A. Endocrine disruptors and testicular function. Metabolism. 2018;86:79–90.
43. Desdoits-Lethimonier C, et al. Human testis steroidogenesis is inhibited by phthalates. Hum Reprod. 2012;27(5):1451–9.
44. Main KM, et al. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ Health Perspect. 2006;114(2):270–6.
45. Jensen MS, et al. Amniotic fluid phthalate levels and male fetal gonad function. Epidemiology. 2015;26(1):91–9.
46. Pan G, et al. Decreased serum free testosterone in workers exposed to high levels of di-n-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): a cross-sectional study in China. Environ Health Perspect. 2006;114(11):1643–8.
47. Mendiola J, et al. Associations between urinary metabolites of di(2-ethylhexyl) phthalate and reproductive hormones in fertile men. Int J Androl. 2011;34(4):369–78.
48. Meeker JD, Calafat AM, Hauser R. Urinary metabolites of di(2-ethylhexyl) phthalate are associated with decreased steroid hormone levels in adult men. J Androl. 2009;30(3):287–97.
49. Araki A, et al. Association between maternal exposure to di(2-ethylhexyl) phthalate and reproductive hormone levels in fetal blood: the Hokkaido study on environment and children's health. PLoS One. 2014;9(10):e109039.
50. Araki A, et al. Prenatal di(2-ethylhexyl) phthalate exposure and disruption of adrenal androgens and glucocorticoids levels in cord blood: The Hokkaido Study. Sci Total Environ. 2017;581-582:297–304.
51. Wagner-Mahler K, et al. Prospective study on the prevalence and associated risk factors of cryptorchidism in 6246 newborn boys from Nice area, France. Int J Androl. 2011;34(5 Pt 2):e499–510.
52. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res. 2008;108(2):177–84.
53. Ormond G, et al. Endocrine disruptors in the workplace, hair spray, folate supplementation, and risk of hypospadias: case-control study. Environ Health Perspect. 2009;117(2):303–7.
54. Carran M, Shaw IC. New Zealand Malayan war veterans' exposure to dibutylphthalate is associated with an increased incidence of cryptorchidism, hypospadias and breast cancer in their children. N Z Med J. 2012;125(1358):52–63.
55. Chevrier C, et al. Maternal urinary phthalates and phenols and male genital anomalies. Epidemiology. 2012;23(2):353–6.
56. Vrijheid M, et al. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup Environ Med. 2003;60(8):543–50.
57. Nassar N, et al. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup Environ Med. 2010;67(9):585–9.
58. Warembourg C, et al. Exposure of pregnant women to persistent organic pollutants and cord sex hormone levels. Hum Reprod. 2016;31(1):190–8.
59. Araki A, et al. Prenatal organochlorine pesticide exposure and the disruption of steroids and reproductive hormones in cord blood: The Hokkaido Study. Environ Int. 2018;110:1–13.
60. Gabel P, et al. The risk of cryptorchidism among sons of women working in horticulture in Denmark: a cohort study. Environ Health. 2011;10:100.
61. Jorgensen KT, et al. Risk of cryptorchidism among sons of horticultural workers and farmers in Denmark. Scand J Work Environ Health. 2014;40(3):323–30.
62. Weidner IS, et al. Cryptorchidism and hypospadias in sons of gardeners and farmers. Environ Health Perspect. 1998;106(12):793–6.
63. Damgaard IN, et al. Persistent pesticides in human breast milk and cryptorchidism. Environ Health Perspect. 2006;114(7):1133–8.
64. Hosie S, et al. Is there a correlation between organochlorine compounds and undescended testes? Eur J Pediatr Surg. 2000;10(5):304–9.

65. Kristensen P, et al. Birth defects among offspring of Norwegian farmers, 1967-1991. Epidemiology. 1997;8(5):537–44.
66. Carbone P, et al. The possible role of endocrine disrupting chemicals in the aetiology of cryptorchidism and hypospadias: a population-based case-control study in rural Sicily. Int J Androl. 2007;30(1):3–13.
67. Biggs ML, Baer A, Critchlow CW. Maternal, delivery, and perinatal characteristics associated with cryptorchidism: a population-based case-control study among births in Washington State. Epidemiology. 2002;13(2):197–204.
68. Carmichael SL, et al. Hypospadias and residential proximity to pesticide applications. Pediatrics. 2013;132(5):e1216–26.
69. Trabert B, et al. Maternal pregnancy levels of trans-nonachlor and oxychlordane and prevalence of cryptorchidism and hypospadias in boys. Environ Health Perspect. 2012;120(3):478–82.
70. Vandenberg LN, et al. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocr Rev. 2009;30(1):75–95.
71. Galloway T, et al. Daily bisphenol A excretion and associations with sex hormone concentrations: results from the InCHIANTI adult population study. Environ Health Perspect. 2010;118(11):1603–8.
72. Minatoya M, et al. Cord blood bisphenol A levels and reproductive and thyroid hormone levels of neonates: The Hokkaido Study on Environment and Children's Health. Epidemiology. 2017;28(Suppl 1):S3–9.
73. Choi H, et al. The association between some endocrine disruptors and hypospadias in biological samples. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2012;47(13):2173–9.
74. Fernandez MF, et al. Bisphenol A and other phenols in human placenta from children with cryptorchidism or hypospadias. Reprod Toxicol. 2016;59:89–95.
75. Chevalier N, et al. A negative correlation between insulin-like peptide 3 and bisphenol A in human cord blood suggests an effect of endocrine disruptors on testicular descent during fetal development. Hum Reprod. 2015;30(2):447–53.
76. Van den Berg M, et al. The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci. 2006;93(2):223–41.
77. Hsu PC, et al. Serum hormones in boys prenatally exposed to polychlorinated biphenyls and dibenzofurans. J Toxicol Environ Health A. 2005;68(17-18):1447–56.
78. Cao Y, et al. Environmental exposure to dioxins and polychlorinated biphenyls reduce levels of gonadal hormones in newborns: results from the Duisburg cohort study. Int J Hyg Environ Health. 2008;211(1-2):30–9.
79. Miyashita C, et al. Sex-related differences in the associations between maternal dioxin-like compounds and reproductive and steroid hormones in cord blood: The Hokkaido Study. Environ Int. 2018;117:175–85.
80. Brucker-Davis F, et al. Cryptorchidism at birth in Nice area (France) is associated with higher prenatal exposure to PCBs and DDE, as assessed by colostrum concentrations. Hum Reprod. 2008;23(8):1708–18.
81. Main KM, et al. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environ Health Perspect. 2007;115(10):1519–26.
82. McGlynn KA, et al. Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. Environ Health Perspect. 2009;117(9):1472–6.
83. Carmichael SL, et al. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere. 2010;80(6):641–6.
84. Lopez-Espinosa MJ, et al. Perfluoroalkyl substances, sex hormones, and insulin-like growth factor-1 at 6-9 years of age: a cross-sectional analysis within the C8 health project. Environ Health Perspect. 2016;124(8):1269–75.
85. Vested A, et al. Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environ Health Perspect. 2013;121(4):453–8.

86. Goudarzi H, et al. The association of prenatal exposure to perfluorinated chemicals with glucocorticoid and androgenic hormones in cord blood samples: The Hokkaido Study. Environ Health Perspect. 2016;125:111–8.
87. Itoh S, et al. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. Environ Int. 2016;94:51–9.
88. Vesterholm Jensen D, et al. No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland. Reproduction. 2014;147(4):411–7.
89. Aschim EL, et al. Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. J Clin Endocrinol Metab. 2004;89(10):5105–9.
90. Radpour R, et al. Association of long polyglycine tracts (GGN repeats) in exon 1 of the androgen receptor gene with cryptorchidism and penile hypospadias in Iranian patients. J Androl. 2007;28(1):164–9.
91. Ferlin A, et al. Androgen receptor gene CAG and GGC repeat lengths in cryptorchidism. Eur J Endocrinol. 2005;152(3):419–25.
92. Wang Q, et al. Association of androgen receptor gene CAG and GGN repeat polymorphism with cryptorchidism: a meta-analysis. Andrologia. 2018;50(3):12909.
93. Wiener JS, et al. Androgen receptor gene alterations are not associated with isolated cryptorchidism. J Urol. 1998;160(3 Pt 1):863–5.
94. Lo Giacco D, et al. ESR1 promoter polymorphism is not associated with nonsyndromic cryptorchidism. Fertil Steril. 2011;95(1):369–71.
95. Wang Y, et al. Analysis of five single nucleotide polymorphisms in the ESR1 gene in cryptorchidism. Birth Defects Res A Clin Mol Teratol. 2008;82(6):482–5.
96. Beleza-Meireles A, et al. Polymorphisms of estrogen receptor beta gene are associated with hypospadias. J Endocrinol Investig. 2006;29(1):5–10.
97. Choudhry S, et al. Genetic polymorphisms in ESR1 and ESR2 genes, and risk of hypospadias in a multiethnic study population. J Urol. 2015;193(5):1625–31.
98. Ban S, et al. Genetic polymorphisms of ESR1 and ESR2 that may influence estrogen activity and the risk of hypospadias. Hum Reprod. 2008;23(6):1466–71.
99. Watanabe M, et al. Haplotype analysis of the estrogen receptor 1 gene in male genital and reproductive abnormalities. Hum Reprod. 2007;22(5):1279–84.
100. Chavez-Saldana M, et al. Single nucleotide polymorphisms associated with nonsyndromic cryptorchidism in Mexican patients. Andrologia. 2018;50(1):12788.
101. Takahashi I, et al. Ala/Thr60 variant of the Leydig insulin-like hormone is not associated with cryptorchidism in the Japanese population. Pediatr Int. 2001;43(3):256–8.
102. Baker LA, et al. The insulin-3 gene: lack of a genetic basis for human cryptorchidism. J Urol. 2002;167(6):2534–7.
103. Kolon TF, et al. Analysis of homeobox gene HOXA10 mutations in cryptorchidism. J Urol. 1999;161(1):275–80.
104. Lu P, et al. Genetic analysis of HOXA11 gene in Chinese patients with cryptorchidism. Andrologia. 2018;50(1):12790.
105. Wang Y, et al. Allelic variants in HOX genes in cryptorchidism. Birth Defects Res A Clin Mol Teratol. 2007;79(4):269–75.
106. Ars E, et al. Further insights into the role of T222P variant of RXFP2 in non-syndromic cryptorchidism in two Mediterranean populations. Int J Androl. 2011;34(4):333–8.
107. Zhou B, et al. The variations in the AXIN1 gene and susceptibility to cryptorchidism. J Pediatr Urol. 2015;11(3):132 e1–5.
108. Ma Q, et al. Diacylglycerol kinase kappa (DGKK) variants and hypospadias in Han Chinese: association and meta-analysis. BJU Int. 2015;116(4):634–40.
109. Zhang K, et al. Steroid 5-alpha-reductase type 2 (SRD5A2) gene V89L polymorphism and hypospadias risk: a meta-analysis. J Pediatr Urol. 2017;13(6):630 e1–9.

110. Samtani R, et al. Hypospadias risk and polymorphism in SRD5A2 and CYP17 genes: case-control study among Indian children. J Urol. 2011;185(6):2334–9.
111. Kojima Y, et al. Single nucleotide polymorphisms of HAAO and IRX6 genes as risk factors for hypospadias. J Urol. 2019;201(2):386–92.
112. Kalfa N, et al. Genomic variants of ATF3 in patients with hypospadias. J Urol. 2008;180(5):2183–8.
113. Beleza-Meireles A, et al. Activating transcription factor 3: a hormone responsive gene in the etiology of hypospadias. Eur J Endocrinol. 2008;158(5):729–39.
114. Fukami M, et al. CXorf6 is a causative gene for hypospadias. Nat Genet. 2006;38(12):1369–71.
115. Ogata T, Fukami M, Wada Y. MAMLD1 (CXorf6) is a new gene for hypospadias. Clin Pediatr Endocrinol. 2008;17(4):87–93.
116. Chen Y, et al. Mutational study of the MAMLD1-gene in hypospadias. Eur J Med Genet. 2010;53(3):122–6.
117. Kalfa N, et al. Mutations of CXorf6 are associated with a range of severities of hypospadias. Eur J Endocrinol. 2008;159(4):453–8.
118. Ohsako S, et al. Expression of xenobiotic biomarkers CYP1 family in preputial tissue of patients with hypospadias and phimosis and its association with DNA methylation level of SRD5A2 minimal promoter. Arch Environ Contam Toxicol. 2018;74(2):240–7.
119. Kurahashi N, et al. Maternal genetic polymorphisms in CYP1A1, GSTM1 and GSTT1 and the risk of hypospadias. Mol Hum Reprod. 2005;11(2):93–8.
120. Qin XY, et al. Individual variation of the genetic response to bisphenol a in human foreskin fibroblast cells derived from cryptorchidism and hypospadias patients. PLoS One. 2012;7(12):e52756.
121. Qin XY, et al. Association of variants in genes involved in environmental chemical metabolism and risk of cryptorchidism and hypospadias. J Hum Genet. 2012;57(7):434–41.
122. Carmichael SL, et al. Joint effects of genetic variants and residential proximity to pesticide applications on hypospadias risk. Birth Defects Res A Clin Mol Teratol. 2016;106(8):653–8.
123. Kalfa N, et al. 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. 2015;68(6):1023–30.
124. Suzuki Y, et al. Screening for mutations of the androgen receptor gene in patients with isolated cryptorchidism. Fertil Steril. 2001;76(4):834–6.
125. Thai HT, et al. The valine allele of the V89L polymorphism in the 5-alpha-reductase gene confers a reduced risk for hypospadias. J Clin Endocrinol Metab. 2005;90(12):6695–8.
126. Sata F, et al. Genetic polymorphisms of 17 beta-hydroxysteroid dehydrogenase 3 and the risk of hypospadias. J Sex Med. 2010;7(8):2729–38.
127. Kalfa N, et al. Polymorphisms of MAMLD1 gene in hypospadias. J Pediatr Urol. 2011;7(6):585–91.

Chapter 5
Endocrine-Distributing Chemicals and Reproductive Function

Atsuko Araki and Tina Kold Jensen

Abstract Exposures to environmental chemicals affecting androgen action (endocrine-disrupting chemicals (EDCs)) are suspected to have a negative impact on male reproductive function by disrupting normal differentiation and development. In this chapter, the literature on the impact of exposure to endocrine-disrupting chemicals on male reproduction will be reviewed. We will specifically address the effects of exposure to organochlorine compounds, perfluorinated alkylate substances (PFAS), phthalates, and phenols on anogenital distance, reproductive hormones in childhood, puberty onset, and semen quality, focusing on prenatal or early exposures during vulnerable time points of development. Generally, anogenital distance (AGD) appears to be a promising, easily obtainable marker of male reproductive health. Maternal exposure to phthalates has consistently been associated with shorter AGD in male offspring, but no consistent associations between PFAS or bisphenol A exposure and AGD have been found. Prenatal exposure to organochlorine pesticides (OCPs) appears to lower children's testosterone concentrations and increase aromatase activity after birth. In addition, prenatal exposure to dioxins and OCPs may delay puberty, whereas exposure to polychlorinated biphenyls (PCBs) accelerates the onset of puberty in boys. Maternal, childhood, or adult phthalate exposure has been associated with lower reproductive hormone concentrations, changed onset of puberty and semen quality. No consistent associations between PFAS or phenol exposure and AGD, reproductive hormones, puberty onset, or semen quality have been found. We suggest that more research is urgently needed focusing on birth cohort studies addressing the adverse effects of exposures during vulnerable time windows during development, e.g., in utero, during early childhood, and puberty. The cohorts should have the necessary size, include biological

A. Araki
Hokkaido University, Center for Environmental and Health Sciences, Sapporo, Japan

T. K. Jensen (✉)
Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense, Denmark
e-mail: tkjensen@health.sdu.dk

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_5

material, focus on multiple exposures, and have long-term follow-up with repeated clinical examinations.

Keywords Anogenital distance · Reproductive hormones · Puberty onset · Testicular dysgenesis syndrome · Semen quality · Organochlorine compounds · Per- and polyfluoroalkyl substances · Phthalates · Bisphenols · Prenatal exposure

5.1 Introduction

In 2002, the World Health Organization (WHO), the International Programme on Chemical Safety (IPCS), United Nations Environment Programme (UNEP), and the International Labour Organization released the "Global Assessment of the State-of-the-Science of Endocrine Disruptors" report [1], which was updated as the "The State of the Science of Endocrine Disrupting Chemicals" in 2012 [2] to address concerns about the potential adverse effects of exposure to chemicals with endocrine-disrupting abilities (EDCs) on humans and wildlife. The Endocrine Society reported that exposure to environmental chemicals may contribute to the increasing incidences of obesity; diabetes mellitus; cardiovascular diseases; infertility; neurodevelopment and certain hormone-sensitive cancers such as the prostate gland, the thyroid, and the brain [3]. In the following we will focus on the adverse effects on male reproductive health.

Male reproductive health may be declining including increased incidence of testicular cancer, reduced semen quality, and increased birth prevalence of hypospadias and cryptorchidism [4]. The testicular dysgenesis syndrome (TDS) hypothesis suggests that these conditions are interlinked and signs of the same underlying unity founded in utero. Exposure to environmental chemicals affecting androgen action during the sensitive window of mascularization from week 8 to 14 of gestation is suspected to have a negative impact on male reproductive function by disrupting normal differentiation and development of the reproductive system. Consequences of such disruptions include compromising the development and function of the testicular Leydig and Sertoli cells [5] (Fig. 5.1 [6]) leading to cryptorchidism, hypospadias, testicular cancer, decreased testosterone production, and impaired spermatogenesis. More recently, decreased anogenital distance (AGD) has been suggested to be part of the TDC syndrome (see later) [7]. In humans, studying the effects of exposures in utero on adult male reproductive function, including semen quality, is challenging due to the long interval between fetal exposure and mature reproductive function.

In the following the literature of exposure to endocrine-disrupting chemicals (organochlorine compounds, perfluorinated alkylated substances (PFAS), phthalates, and phenols) on anogenital distance, reproductive hormones in childhood, puberty onset, and semen quality will be reviewed.

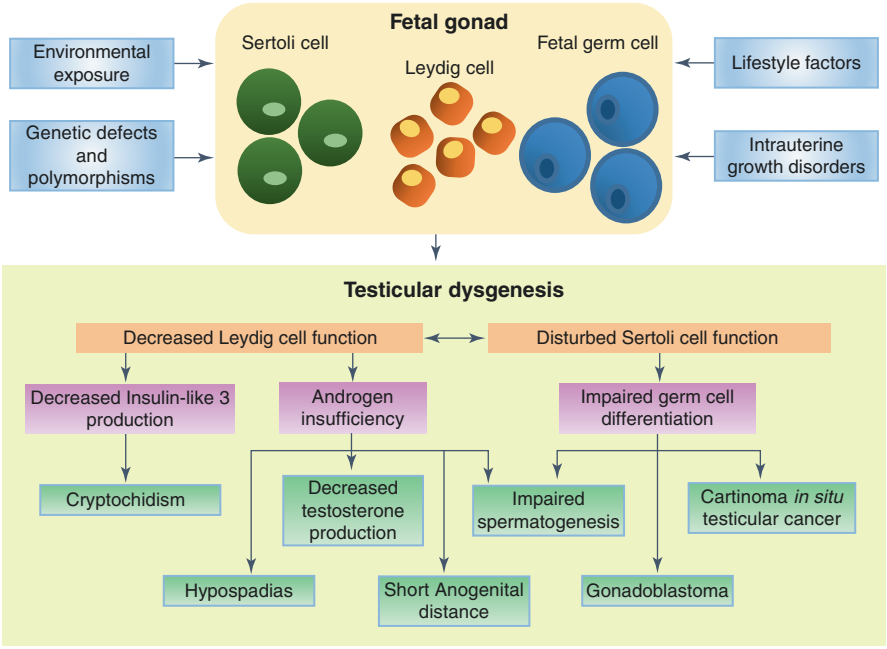


Fig. 5.1 Testicular dysgenesis syndrome. Multiple genetic and environmental factors ensure appropriate fetal testicular development, but adverse environmental exposures, genetic aberrations, lifestyle factors, and intrauterine growth disorders can all result in testicular dysgenesis. Testicular dysgenesis in early fetal life influences Leydig cells, Sertoli cells, and the niche supporting fetal germ cells. This influence can result in intermediate phenotypes (pink boxes), some of which can have late symptoms and consequences (green boxes). (Adapted from Juul et al. [6])

5.2 Evidence from Epidemiological Studies

5.2.1 Anogenital Distance as a Marker of Exposure

Anogenital distance (AGD; distance from anus to genitals) is routinely used in animal toxicology studies, and is sensitive to anti-androgenic exposure. In rodents, AGD has been shown to reflect the amount of androgen to which a male fetus is exposed in early development; higher in utero androgen exposure results in longer AGD. In male rodents, shortened AGD persists into adulthood [8] and predicts compromised reproductive function (reduced testis size) in the mature male [9].

5.2.1.1 AGD Definition

Interestingly, AGD has been measured in humans and may be a part of the TDS syndrome [5]. This is also in line with the theory that TDS outcomes result from a disturbance in the Sertoli cell and Leydig cell differentiation during fetal life,

leading to impaired testosterone production and decreased virilization. There is indirect evidence for a link between TDS conditions and AGD; AGD is shorter in patients with infertility [10], poor semen quality [11], lower testosterone concentrations [12], cryptorchidism and hypospadias [13]. This evidence suggests that shortened AGD is part of the TDS syndrome [7]. Because it can be measured in all boys, it is a more sensitive marker of genital development than the birth prevalence of cryptorchidism or hypospadias, which are found in less than 10% of newborns, thus requiring large study populations.

AGD can easily be measured by the use of a Vernier caliper; the shorter AGD measurement is measured from the center of anus to the posterior base of scrotum (AGDas) and the longer from the center of anus to the cephalad insertion of the penis (AGDap).

5.2.1.2 Organochlorine Compounds

Organochlorine compounds include organochlorine pesticides (OCP), polychlorinated biphenyl (PCB) compounds, and dioxins. Due to their highly persistent properties, the Stockholm Convention has regulated their use. OCP are chlorinated hydrocarbons, used extensively in the 1940s for agriculture and pesticide control. Most OCP were banned in the US, Europe, and many other countries in the early 1970s [14]. Although the Stockholm Convention has issued an exemption for the production and public health, use of dichlorodiphenyltrichloroethane (DDT) is still prominent to control vector-borne diseases. PCBs were used as a non-flammable insulate and heat stabilizer in industries until the 1970s. Dioxins and dioxin-like compounds are mostly the by-products of various industrial processes. Most OCP and PCBs have been prohibited for over 30 years; however, due to their long half-life, they are still detected in the blood of most individuals including pregnant women [15, 16].

In the U.S., a smaller study including 37 male offspring indicated reduced anogenital distance at higher *p*, *p*′-DDE exposure [17]. However, a larger study, among 781 mother–child pairs in Chiapas, Mexico, of which 29% reported living in DDT-sprayed homes, indicated no association between *p*, *p*′-DDE exposure and anogenital distance or penile length, suggesting that even high exposure to *p*, *p*′-DDE does not seem to have a significant impact on these outcomes in humans [18]. A smaller Mexican study with repeated AGD measurements found an association between maternal PCBs and AGD in 74 boys, whereas no associations were found for prenatal DDT exposure [19].

In a Spanish mother–child cohort, POP concentrations were measured in pregnant women, and AGDas was recorded in 43 offspring at 18 months of age. Anogenital index was calculated as AGD divided by weight and found to be inversely associated with lipid-adjusted concentrations of PBDE-99 and PBDE-153 but not with PCB congeners [20]. In a Danish mother–child cohort, no consistent association between prenatal exposure to the pesticide metabolites 3-phenoxybenzoic

acid (3-PBA), 3,5,6-trichloro-2-pyridinol (TCPY), 2,4-Dichlorophenoxyacetic acid (2,4-D), and dialkyl phosphates (DAPs) and AGD was found [21].

5.2.1.3 PFAS

PFASs compose a group of chemicals which are considered persistent organic pollutants because of their resistance to biodegeneration. They are mainly used as surfactants in a wide range of consumer products (e.g., paint and lacquers, carpets, impregnated outdoor clothing, food packaging) because of their water and soil-repellent properties. Compounds composed of chains of eight carbons, perfluorooctane sulfonic acid (PFOS), and perfluorooctanoic acid (PFOA) have been the most extensively produced. Due to their persistent properties, PFOS is listed in Annex B of the Stockholm Convention in 2009, and PFOA is listed in Annex A in 2019 [14].

To the best of our knowledge only one human study has addressed the association between maternal exposure to PFAS and AGD in the offspring. PFOS, PFHxS, PFNA, and PFDA were associated with a decreased AGD in *girls*, whereas no associations were reported in boys [22].

5.2.1.4 Phthalates

Phthalates are high volume production chemicals, and their applications in industrial production are determined by molecular weight. High molecular weight phthalates, such as di-(2-ethylhexyl) phthalate (DEHP) and diisononyl phthalate (DiNP), are used as plastic softeners in numerous polyvinyl chloride (PVC) products, like rain wear and shoes, flooring, food packaging, toys, and medical devices. Low molecular weight phthalates, such as dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalate (DBP), are primarily used as solvents in personal care products, lacquers, insecticides, and in coatings [23]. Humans are mainly exposed to environmental phthalates through ingestion (high molecular weight phthalates), indoor air, and dermal contact (low molecular weight phthalates) and inhalation [24]. The biological half-life of phthalates is in the range of hours to days, and they do not accumulate in the body; however, phthalate metabolites are present in urine in the majority of the human population indicating continuous exposure [25–27].

A U.S. study, which included 134 mother–son pairs, was the first investigation to explore AGD in humans. The study found a significant association between maternal exposure to several phthalates measured in urine and reduced AGD in the male offspring [28]. Later results found an inverse association between maternal urine DEHP exposure and AGD and penile size [29]. A recent review [30] and meta-analysis, which included 10 studies [31], reported that prenatal exposure to DEHP was significantly associated with shorter AGD. In addition, urinary monobutyl phthalate (MBP), monoethyl phthalate (MEP), and mono-*i*-butyl phthalate (MiBP) were found to be associated with short AGD (AGDas).

5.2.1.5 Bisphenol A (BPA)

Bisphenol A (BPA) is a high production volume chemical that is widely used in the manufacture of consumer products such as polycarbonate plastics, epoxy resin liners of canned foods, some dental sealants and composites, and thermal receipts. Due to its widespread use in consumer products, exposure to BPA is ubiquitous [32].

Many rodent studies have demonstrated that maternal BPA exposure decreased AGD in male offspring [33]; however, few human studies have been performed. Most studies have been conducted in China, where exposure concentrations are high. An occupational cohort study revealed that maternal exposure to BPA during pregnancy was associated with shortened AGD in male offspring [34]. However, few women were occupationally exposed to high doses of BPA (n = 18). In a Chinese cohort study, among 655 mother–son pairs, mothers with detectable BPA in urine in gestational week 12–16 gave birth to boys with shorter AGDap at 6 and 12 months of age [35]. These findings were not replicated in a Chinese study among 137 boys; i.e., no significant association between maternal BPA and AGD was found. However, data were not provided, and the measurement of AGD was not specified [36]. These findings are in accordance with findings from Canadian study among 198 boys, where no significant associations between maternal BPA exposure and AGD were reported [37]. A study conducted in Cypress measured BPA in cord blood, which may be difficult to compare to urine measurements, which is the gold standard. No significant correlations between cord blood BPA concentrations and AGD were found; however, a significant negative correlation between AGDas and cord blood BPA concentrations above the 90th percentile was found in 72 boys [38].

5.2.2 Environmental Chemical Exposure and Reproductive Hormones

5.2.2.1 Reproductive Hormone In Utero and Early Life

During early gestation, the reproductive organs differentiate into the testis and ovaries, and molecular stimuli may curtail these processes. In men, Sertoli cells secrete testosterone and cross-talk with Leydig cells, which produce virilization. Thus, during early development, exposure to proper concentrations of hormones is essential. Exposure to exogenous substances with endocrine-disrupting properties can mimic or antagonize these and other hormonal systems to change the developmental trajectory [3]. As a result, we looked at studies that examined the effect of exposure to EDCs on reproductive hormones at birth and during childhood. The results of birth cohort and longitudinal studies together with cross-sectional data at baseline are summarized in each section and Table 5.1.

Table 5.1 Prenatal exposures and reproductive hormones

Country, Year	Participants (number of samples)	Exposures	P4	T	E2	T/E2	SHBG	LH	FSH	Inhibin B	INSL3	Others	Ref.
Chlorinated chemicals													
Germany, 2008	Industrial area birth cohort (n = 104)	Prenatal dioxins		↓	↓								[41]
France, 2016	PELAGIE	Prenatal PCBs		↓		↓	↑						[16]
	(n = 282)	Prenatal α-endosulfan and HCE		→	↑	↓	↑						
Japan, 2018	Hokkaido Study: Sapporo Cohort (n = 257)	Prenatal dioxins (M)	→	→	→	→	→	→	→	↓	→		[112]
		Prenatal OCPs	→	↓	→	↑	↓	→	→	→	→	DHEA↑, T/androstenedione↓, prolactin↓	[42]
USA, 2017	CHAMACOS	Prenatal BDE-153		↑				↑	↑				[43]
	(males n = 234)	Prenatal PCBs		→				→	↑				
		Prenatal DDT		↓				↓	→				
		Prenatal DDE		→				↓	→				
Taiwan, 2005	Males with Yucheng mothers (cases n = 60, controls n = 61)	Cases compared to controls		→	→	↓		→	→			T/FSH↓, E2/FSH↑	[39]
Taiwan, 2005	Females with Yucheng mothers (cases n = 27, controls n = 21)	Cases compared to controls		→	→	→		→	↑				[40]

(continued)

Table 5.1 (continued)

Country, Year	Participants (number of samples)	Exposures	P4	T	E2	T/E2	SHBG	LH	FSH	Inhibin B	INSL3	Others	Ref.
Denmark, 2012	Faroe Islands birth cohort (males $n = 428$)	Prenatal PCBs		↓			↑	↓					[113]
		Postnatal (14 years of age) PCBs		→			↑	→					
PFAS													
Denmark, 2015	Cryptorchidism (cases $n = 270$), hypospadias (cases $n = 75$, controls $n = 300$)	Prenatal PFOS	→	↓							↓		[45]
Japan, 2016	Hokkaido Study: Sapporo Cohort ($n = 257$)	Prenatal PFOS (M)	↓	→	→	↓	→	→	→	↓	↓		[46, 47]
		Prenatal PFOA (M)	→	→	→	→	→	→	→	↑	→	DHEA↓	
		Prenatal PFOS (F)	↓	→	→	→	↓	→	→	→	→	Prolactin ↓	
		Prenatal PFOS	→	→	→	→	→	→	→	→	→	DHEA↑, cortisol↓, cortisone↓	
UK, 2015	ALSPAC (female $n = 75$)	Prenatal PFOS, PFOA, and PFHxS		↑			→						[48]
Denmark, 2013	Danish population-based cohort (males $n = 176$)	Prenatal PFOA		→	→	→	→	↑	↑	→	→		[106]

Phthalates												
Taiwan, 2011	Birth cohort (n = 155)	Prenatal 3rd trimester DEHP (F)		↓	→	↓						[49]
Japan, 2014, 2016	Hokkaido Birth Cohort Study: Sapporo cohort (n = 257)	Prenatal 2–3rd trimester DEHP (M)	↓	→	→	↓	→	→	→	↓	↓		[50, 51]
		Prenatal 2–3rd trimester DEHP	↓	→	→	→	→	→	→	→	→	Cortisol↓, cortisone↓, cortisol/cortisone↓, glucocorticoid/adrenal androgen↓, DHEA/androstenedione↑	
Denmark and Finland, 2006	Danish–Finnish prospective cohort (cryptorchidism cases n = 62, controls n = 68)	Postnatal (1–3 months) phthalate metabolites		↓			↑	↑				LH/T↑	[52]
Taiwan, 2017	TMICS	Prenatal 3rd trimester DEHP	↓ (F)	→	→								[53]
	(n = 193)	Postnatal 2, 5, 8, and 11 years of age DEHP	↓ (F)	↓ (M)	→								
Mexico, 2017	ELEMENT	Prenatal 3rd trimester phthalate metabolites (M)		→	↑		↑			→			[54, 55]
	(n = 229)	Prenatal 3rd trimester phthalate metabolites (F)		↑	→		→			→		DHEA-S↑	

(continued)

Table 5.1 (continued)

Country, Year	Participants (number of samples)	Exposures	P4	T	E2	T/E2	SHBG	LH	FSH	Inhibin B	INSL3	Others	Ref.
Denmark, 2013	Copenhagen Puberty Study	Postnatal (5–19 years of age) MBP (F)		→ (13 y)				→ (13 y)	→ (13 y)			DHEAS↓, Δ4-androstenedione↓	[81]
	Longitudinal study 168 children (males *n*= 84 and females *n* = 84)	Postnatal (5–19 years of age) MBP (M)		↑ (13 y)				→	→			DHEAS↓ (11 y)	
Bisphenols													
France, 2012	Prospective cohort (cryptorchidism cases *n* = 46, controls *n* = 106)	At birth BPA (M)		↑	→		→	→	→	↑			[56]
Japan, 2014	Hokkaido Study: Sapporo Cohort (*n* = 278)	At birth BPA (M)	↑	↑	→	→	→	→	→	→	→		[57]
China, 2017	Polluted area and control area (males *n* = 137)	At birth BPA		↓	→	↓							[36]
Czech Republic, 2018	Prospective birth cohort (*n* = 27)	At birth BPA, BPF, BPS, BPAF										No associations	[114]

BPA bisphenol A, *DDE* dichlorodiphenyldichloroethylene, *DDT* dichlorodiphenyltrichloroethane, *DHEA* dehydroepiandrosteone, *DHEA-S* dehydroepiandrosteone-sulfonate, *E2* Oestradiol, *F* Female, *FSH* follicle-stimulating hormone, *INSL3* insulin-like factor-3, *LH* luteinizing hormone, *M* male, *OCP* organochlorine pesticide, *PBDE* polybrominated diphenyl ether, *PCB* polychlorinated biphenyl, *PFHxS* perfluorohexanesulfonic acid, *PFNA, PFOA* perfluorooctanoic acid, *PFOS* perfluorooctanesulfonic acid, *SHBG* steroid hormone binding globulin, *T* testosterone, *T/E2* testosterone/oestradiol ratio, *y* year-old

↑, positive association, ↓, inverse association, →, no association

5.2.2.2 Organochlorine Compounds

A total of eight birth cohort studies were identified, as shown in Table 5.1. Two from Taiwan targeted Yucheng mothers, who were exposed to rice oil contaminated accidentally with PCBs and PCDFs in 1978–1979 [39, 40]. Children of Yucheng mothers aged more than 13 years showed significantly lower testosterone/oestradiol (T/E2) ratio and T/follicle-stimulating hormone (FSH) and higher E2/FSH ratio among boys of control group (non-Yucheng mothers) [39]. Girls of Yucheng mothers, significantly higher E2 and FSH concentrations were observed when compared control group of non-Yucheng mothers [39, 40]. Although these studies did not measure the blood concentrations of PCBs and dioxins, the lower T/E2 ratio among Yucheng children is in line with the results from other studies. In six studies chlorinated chemicals in either maternal or cord blood were measured and reproductive hormone concentrations in cord or child blood were assessed. Increased maternal blood or cord blood concentrations of PCBs and OCPs were consistently associated with lower testosterone concentrations, along with lower T/E2 ratio (or higher E2/T ratio), a marker of aromatase activity. These results were found at birth in Germany (dioxins) [41], France (PCBs and OCPs) [16], and Japan (OCPs) [42], at age 12 in the US (DDT) [43], and at age 14 in Denmark (PCBs) [44]. A U.S. study, however, reported that increased maternal BDE-153 exposure increased testosterone concentrations among boys at age 12 years [43].

5.2.2.3 PFAS

Few studies have examined the association between prenatal PFAS exposure and reproductive hormone concentrations among children. A Danish study compared cryptorchidism and hypospadias with matched controls and measured hormone concentrations in the amniotic fluid. PFOS concentrations in amniotic fluid were inversely correlated with testosterone and insulin-like factor 3 (INSL3) concentrations among all children [45]. Maternal PFOS concentrations and lower concentrations of progesterone T/E2, inhibin B, and dehydroepiandrosterone (DHEA) in cord blood was observed among boys in a birth cohort in Japan [46, 47]. The findings suggest that PFOS exposure resulted in undermatured testis. Whereas a positive association between PFOA and inhibin B and DHEA in cord blood among boys in a Japanese cohort suggested the opposite association between PFOS and PFOA [46, 47]. Moreover, prenatal PFOS exposure was positively associated with testosterone concentrations in child blood at 15 years of age among girls [48]. Thus, the effects of PFOS and PFOA on reproductive hormone concentrations may differ according to age and be modified by sex.

5.2.2.4 Phthalates

Two studies from Taiwan and Japan reported that concentrations of the most dominant phthalate, DEHP, or its metabolite mono(2-ethylhexyl) phthalate (MEHP) in mothers urine during pregnancy were inversely related to children's T/E2 ratio at

birth, which may reflect upregulation of aromatase cytochrome P450 (CYP19) [49, 50]. The Japanese study reported reduced concentrations of progesterone at birth but increased concentrations of DHEA/androstenedione, which may reflect the downregulation of hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (HSD3B1) [51]. Moreover, reduced concentrations of testosterone were also observed in the blood of boys aged 1–3 months [52]. Another Taiwan study measured DEHP metabolites both in mother and child urine and found an association between higher prenatal DEHP concentrations with reduced concentrations of female children's progesterone at a later age of 2, 5, 8, and 11 [53]. In the same cohort, child urinary metabolites of DEHP concentrations at 2, 5, 8, and 11 years of age were inversely correlated with testosterone among boys and progesterone among girls [53]. These results indicate that phthalate exposure may decrease concentrations of testosterone, progesterone, and T/E2 ratio, suggesting the upregulation of CYP19. However, a Mexican study found that higher exposure to phthalate metabolites during pregnancy was associated with increased concentrations of oestradiol among boys and testosterone and DHEA-sulfate among girls [54, 55] aged 8–13 years of age.

5.2.2.5 BPA

Three studies examined both BPA exposure concentrations and reproductive hormones at birth. A French and a Japanese study reported a positive association between maternal BPA and testosterone and progesterone [56, 57] in the male offspring, whereas a Chinese study reported an inverse association between prenatal BPA exposure and testosterone [36] in new-born boys.

5.2.3 Environmental Chemical Exposure and Onset of Puberty

5.2.3.1 Onset of Puberty

Hormones play an important part in defining the optimal conditions for human reproductive life to start [58]. Another critical period of development with a sensitive window of exposure to EDCs is puberty, during organs maturation [59]. To evaluate the onset of puberty most studies evaluate Tanner stage [60]. For girls, breast developmental and pubic hair stages, as well as menarche, are determined. For boys, genital and pubic hair stages and testicular volume were mainly used. The current evidence and longitudinal studies on exposure to environmental chemicals and their associations with the onset of puberty are collected and summarized in Table 5.2.

Table 5.2 Exposures to EDCs and onset of puberty

Country, Year	Design and participants	Exposure and outcome measurements	Breast development	Pubic hair	Axillary hair	Genital stage	Menarche	Testicular volume	Others	Ref.
Chlorinated chemicals										
The Netherlands, 2008	Longitudinal cohort ($n = 33$)	Prenatal PCDD/PCDF	Delay (F)	→	→	→	→			[61]
Denmark, 2013	Danish population-based cohort established in 1988–1989 (females 20 years of age $n = 436$)	Prenatal *p,p′*-DDE, HCB					→		Antral follicle number ↓	[62]
UK, 2016	ALSPAC females (menarche age <11.5 years as cases $n = 218$, controls $n = 230$)	Prenatal 9 OCPs					→			[63]
USA, 2015	Breast Cancer and Environment Research Centers (BCERC) project (females 6–8 years old, $n = 645$)	Postanal 8–6 years of age ΣPCB	Delay (cross-sectional)							[64]
		Postnatal 8–6 years of age ΣPCBs, PBDEs, OCPs	Delay (2, 5 years follow-up)	Delay (2, 5 years follow-up)						
Russia, 2011	Prospective cohort (males $n = 489$)	Prenatal PCB		→		Delay		→		[65]
		Prenatal TEQ		→		→		→		
Russia, 2011	Prospective cohort (males $n = 499$)	Postnatal 8–9 years of age TEQ		→		→		Delay		[66]
		Postnatal 8–9 years of age 2,3,7,8-TCDD (8–9 years)		→		→		Delay		

(continued)

Table 5.2 (continued)

Country, Year	Design and participants	Exposure and outcome measurements	Breast development	Pubic hair	Axillary hair	Genital stage	Menarche	Testicular volume	Others	Ref.
Russia, 2014	Prospective cohort (males 8–9 years old $n = 350$)	Postnatal 8–9 years of age HCB		Delay		→		Delay		[67]
		Postnatal 8–9 years of ageβ-HCH, *p,p′*-DDE		→		→		→		
Russia, 2016	Prospective cohort (males $n = 473$)	Postnatal 8–9 years of age TEQ	TEQ	Delay (17–18 years)						[68]
		Postnatal 8–9 years of age Non-DL PCB		Early (17–18 years)						
PFAS										
Denmark, 2013	Danish population-based cohort established in 1988–1989 (females $n = 343$)	Prenatal PFOA					Delay			[70]
UK, 2011	ALSPAC (females menarche age <11.5 years old as cases $n = 218$, control $n = 230$)	Prenatal PFAS					→			[71]
Phthalates										
Taiwan, 2015	TMICS birth cohort study	Prenatal 3rd trimester MEHP, total DEHP						→	Reduced uterus size (F)	[73]
	($n = 437$ at baseline)	Prenatal 3rd trimester MBzP	↑ (F)					→	Bone age/ chronological age ratio (F)	

Mexico, 2017	ELEMENT birth cohort (females 8–13 years old, $n = 120$)	Prenatal 3rd trimester MEHP	Delay	Early						[74, 75]
	(males 8–14 years old, $n = 109$)	Prenatal 3rd trimester MBzP		Delay						
USA, 2010	Breast Cancer and Environment Research Centers (BCERC) project	postnatal (6–8 years of age) high molecular weight phthalate	→	Delay						[76]
	Females aged 6–8 years $n = 1151$ (baseline of [77])	postnatal (6–8 years of age) low molecular weight phthalate	Early	→						
USA, 2010	BCERC project (females 6–8 years old, $n = 1151$)	Postnatal (6–8 years of age) high molecular weight phthalate	→	Delay						[77]
USA, 2017	BCERC project (females 6–8 years old, $n = 1051$)	Postnatal (6–8 years of age) MCPP					Delay			[78]
Denmark, 2012	Copenhagen Puberty Study (baseline of [81]) (males $n = 555$)	Postnatal 6.07–19.83 years of age phthalate metabolites		→		→				[79]
	(females $n = 725$)	Postnatal 5.6–19.1 years of age (phthalate metabolites except MEP)	→	Delay					No association with precautious puberty	[80]

(continued)

Table 5.2 (continued)

Country, Year	Design and participants	Exposure and outcome measurements	Breast development	Pubic hair	Axillary hair	Genital stage	Menarche	Testicular volume	Others	Ref.
Denmark, 2013	Copenhagen Puberty Study (longitudinal, $n = 168$)	Postnatal follow-up for 5 years per 6 months 14 urinary phthalate	→ (F)	→ (F)						[81]
		Postnatal follow-up for 5 years per 6 months MBP		Early (M)		→ (M)				
Taiwan, 2015	Prospective cohort at baseline 9.7 ± 2.2 years (males $n = 222$; females $n = 208$)	Postnatal 9.7 years of age MnBP		Delay (M)						[82]
	(females $n = 208$)	Postnatal 9.7 years of age MEHP, MEHHP, and MEOHP	Early (F)				Early (F)			
Phenol										
USA, 2017	BCERC project (females 6–8 years old, $n = 1051$)	Postnatal (6–8 years of age) 2,5-dichlorophenol					Early			[78]

B Tanner scale breast development stage, *DDE* dichlorodiphenyldichloroethylene, *DDT* dichlorodiphenyltrichloroethane, *DEHP* di(2-ethylhexyl) phthalate, *DL-PCB* dioxin like-polychlorinated biphenyl, *G* Tanner scale genitals, *HCB* hexachlorobenzene, *HCH* hexachlorocyclohexane, *MBzP* monobenzyl phthalate, *MCPP* mono-(3-carboxypropyl) phthalate, *MEHHP* mono(2-ethyl-5-hydroxyhexyl) phthalate, *MEP* monoethyl phthalate, *MEHP* mono(2-ethylhexyl) phthalate, *MEOHP* mono (2-ethyl-5-hydroxyhexyl) phthalate, *MnBP* mono-*n*-butyl phthalate, *MMP* monomethyl phthalate, *OCP* organochlorine pesticide, *PCDD* polychlorinated dibenzo-*p*-dioxins, *PCDF* polychlorinated dibenzofuran, *PBDE* polybrominated diphenyl ether, *PCB* polychlorinated biphenyl, *PFAS* perfluorinated alkylated substances, *PFOA* perfluorooctanoic acid, *PH* Tanner scale pubic hair, *TEQ* toxic equivalent

5.2.3.2 Organochlorine Compounds

The adverse effects of exposure to organochlorine compounds such as PCBs, dioxins, and chlorinated pesticides have been extensively studied. In eight prospective studies, three were birth cohorts from the Netherlands, Denmark, and the UK (see Table 5.2). The Dutch study examined prenatal and lactational dioxin exposure and found that higher exposure delayed initiation of breast development among girls, although the sample size was only 33 [61]. In Denmark and the UK concentrations of organochlorine pesticides in maternal blood were measured and no association with age of menarche [62, 63] was found. In an American study, blood concentrations of PCB and OCP in girls aged 7–8 years [64] were associated with delayed onset of breast and pubertal hair development (Tanner stage 2) [64]. Four studies from Russia reported on the same population of boys [65–68]. The blood concentrations of PCBs, dioxins and organochlorine pesticides in these boys at 8–9 years of age were measured. Relatively consistent results were obtained indicating that higher concentrations of dioxins, dioxin-toxicity equivalents (TEQ), and hexachlorobenzene (HCB) were associated with a delayed onset of puberty, while an earlier onset was observed for boys with higher concentrations of non-dioxin like PCB. Although not listed in Table 5.2, two cross-sectional studies from Belgium and Kazakhstan [69] also reported an association between higher concentrations of PCBs, dioxins, and OCPs and delayed pubertal development among both boys and girls, which is consistent with the findings from longitudinal studies.

5.2.3.3 PFAS

Only two prospective studies examined the association between prenatal exposure to PFAS and age of puberty, both studies focused on females [70, 71]. A study from Denmark found that higher concentrations of prenatal PFOA were associated with delayed onset of menarche [70], whereas a UK study found no association [71]. The results of a cross-sectional study conducted in the Mid-Ohio Valley in the US suggested that PFOA and PFOS were associated with a later age of puberty in both boys and girls [72].

5.2.3.4 Phthalates

In a Taiwanese study, higher concentrations of DEHP in maternal urine were associated with girls at 8 and 11 years of age of reduced uterus size, while higher concentrations of butyl benzyl phthalate (BBzP) were associated with increased bone age/chronological age ratio of girls at 8 and 11 years of age [73]. Among Mexican girls, higher exposure to DEHP in utero was associated with the earlier development of pubic hair and delayed breast development at 8–13 years of age [74], whereas a higher exposure to DEHP delayed pubic hair development among boys at 8–14 years

of age [75]. Higher urinary phthalate metabolites in girls at 6–8 years of age were associated with delayed onset of puberty both cross-sectional at baseline and after 2 or 5 years follow-up in American girls [76–78]. In contrast, in a cross-sectional setting, increased concentrations of phthalates delayed puberty among Danish girls (5–19 years of age), whereas no association was found in boys (6–19 years of age) [79, 80]. However, at 5 years follow-up, higher exposure to DBP was related to an early age of puberty among boys but not in girls [81]. Finally, a Taiwan study examined phthalates in urine among 9 year olds and found that DBP delayed pubic hair development in boys, whereas DBP, DMP, DEP, and DEHP exposures accelerated breast and menarche onset in girls after 1.5 years follow-up [82].

5.2.3.5 BPA and Phenols

A prospective U.S. cohort among girls aged 6–8 years urinary concentrations of 2,5-dichlorophenol at baseline was associated with earlier onset of menarche after 2 or 5 years of follow-up [78]. One review examined previous knowledge of the impact of BPA on puberty [83]. The authors concluded that currently available data do not allow the establishment of a clear role of BPA in pubertal development, as the results were conflicting. Moreover, most of the performed studies were cross-sectional or case–control studies comparing precocious puberty children to children without precocious puberty children. As a result, no conclusions on the effects of BPA exposure on puberty onset can be drawn.

5.2.4 Semen Quality

5.2.4.1 Definition and Trends

Semen quality is assessed by concentration, volume, total sperm count (concentration × volume), morphology and motility. In addition, more sophisticated measures of DNA fragmentation, sperm apoptosis and sex-ratio ect. can be performed. Semen quality is an important marker for couple fecundity (ability to conceive) [84], and some studies have suggested that semen quality may be a marker for subsequent morbidity and mortality [85, 86]. A possible decline in semen quality has prompted discussion after a meta-analysis in 1992 suggested a decline of 0.9 ml/ml/year during a 50-year period from 1940 to 1990 [87]. Interestingly, a newly published meta-analysis including studies from 1973 to 2011 reported a similar yearly decline of 0.7 mill/ml [88].

The literature in this field is large, and we have therefore included reviews and meta-analyses when available and focused on studies examining the effect of fetal exposure, as the TDS hypothesis suggests that these are considered most relevant.

5.2.4.2 Organochlorine Compounds

An extensive number of epidemiological studies have addressed the possible effects of exposure to POPs on male reproductive health, but the results are conflicting (reviewed by [89]). Overall, studies of exposure to PCBs during adulthood indicate some association between PCB and lower sperm motility and to some extent decreased sperm DNA chromatin integrity [89]. However, two Faroese studies among high exposed young men and fertile men found no association with semen quality [90, 91].

In high exposed South African and Mexican populations, an inverse association between *p*, *p*′-DDE exposure and semen volume, total sperm count, and computer-assisted sperm analysis mean motility were reported [92, 93]. However, the adverse effects of low exposure to *p*, *p*′-DDE on sperm motility have been contradictory [89]. Some studied have suggested a positive association between *p*, *p*′-DDE exposure and sperm concentration, whereas several studies have suggested that *p*, *p*′-DDE is not related to sperm morphology and sperm DNA integrity [89].

Most studies have been cross-sectional and investigated exposure during adulthood. Only a few studies have been able to evaluate, whether intrauterine exposure to POPs has long-term consequences for male reproductive health with measurable effects on semen quality in adulthood. A Danish study included 176 male offspring from a Danish cohort of pregnant women, who participated in a study in 1988–1989. Results suggested that in utero exposure to PCB and DDE was not significantly associated with semen quality measures [44]. Among 39 sons and mothers exposed to dioxin after the devastating, industrial accident in Seveso, Italy in 1976 and 58 unexposed [94] average sperm counts were almost halved in the exposed group and the effect was most pronounced among breastfed men.

5.2.4.3 Phthalates

Many Chinese studies have addressed the adverse effect of phthalate exposure on semen quality. In 1040 Chinese men from an infertility clinic, higher MBP was associated with low sperm concentration and total sperm count [95]. Higher exposure to MEHP increased the percentage of abnormal heads. A subset of the men had phthalates measured in semen, and higher concentrations and MEHP and monobenzyl phthalate (MBzP) reduced sperm motility parameters and semen volume [96]. In older Chinese men, higher urinary MEP was significantly associated with a decreasing percentage of normal morphology [97].

Two meta-analyses of the impact of phthalate exposure on semen quality have been performed [30, 98]. The first from 2015 included 14 studies and found that urinary MBP and MBzP were associated with reduced sperm concentration. MBP and MEHP were inversely associated with motility [30]. No associations were observed between MEP and any semen parameters. A meta-analysis from 2017 [98]

included 15 studies. Many of the individual study results were not significant, which may be due to small sample size and large both inter- and intra-individual variation in semen quality. Overall, the association between increased DBP exposure and decreased semen quality, specifically sperm concentration, was robust, whereas moderate evidence of an association between increased DEHP and decreased semen quality, particularly for sperm concentration was suggested. Given the consistency across studies for morphology, the relationship between DiNP exposure and sperm parameters was considered moderate. The relationship between DiBP exposure and semen parameters was considered slight, whereas moderate evidence of an adverse effect of BBzP exposure specifically for motility was suggested [98].

Two studies have assessed maternal phthalate exposure and subsequent semen quality in her son [99, 100]. These are of special interest, as this is the relevant exposure window. DiNP metabolites in maternal serum from 12 weeks of pregnancy were analyzed and semen quality assessed among 112 adolescent Swedish sons. Higher prenatal exposure to DEHP and DiNP was associated with lover testicular size and semen volume, whereas no association with semen concentration was found. Among 185 young Australian men, maternal serum phthalate metabolite concentrations of mono-isononyl phthalate (MiNP) and DEHP and DiNP metabolites were negatively correlated with testis volume. It is difficult to draw conclusions from these two studies, as phthalates have a short half-life and were measured in serum and not the golden standard urine, but they suggest that prenatal exposure to DEHP and DiNP may affect testis development and thereby adult testis size.

5.2.4.4 PFAS

In a review [101], nine studies were identified investigating the association between PFAS exposure and semen characteristics. The findings for semen volume, sperm concentration, and total sperm count were inconsistent. Two studies found serum concentrations of PFAS to be associated with sperm morphology [102, 103]. However, in an American study PFAS concentrations were not consistently associated with overall sperm morphology [104, 105] but with makers of immature sperms with tail deficiencies. A few studies reported on the possible associations between PFAS exposure and sperm DNA integrity and apoptotic markers. No strict conclusions can be drawn from these studies [101].

Among 169 young Danish men, whose pregnant mothers were recruited in 1988–1989, prenatal PFOA exposure was associated with lower adjusted sperm concentration and total sperm count, while no associations were found for PFOS [106]. This is interesting, as these men are unselected and exposed during the vulnerable period in utero. In addition, PFAS have long half-lives and therefore maternal concentrations of PFAS represent the exposure during pregnancy.

5.2.4.5 BPA

In a review from 2016, five studies were identified [107]. Li and colleagues explored the association between urinary BPA concentrations and semen parameters among 218 factory workers from four regions in China [108]. Results indicated a negative association between urinary BPA concentrations and sperm concentration, total sperm count, sperm vitality, and sperm motility. However, results only remained significant for sperm concentration for non-occupationally exposed men [108]. In a cross-sectional Danish study, among young men from the general population, urinary BPA was inversely associated with progressive sperm motility [109]. One study among infertile men found that urinary BPA concentrations were negatively associated with sperm concentration, normal morphology, and sperm DNA damage and lower percentage progressively motile sperm, whereas two other studies, of fertile men or men trying to conceive, found no association between urinary BPA and semen quality [107].

To the best of our knowledge, no studies have examined the effect of prenatal BPA exposure on semen quality.

5.3 Challenges for Future Studies

Most studies of the association between endocrine-disrupting chemicals (EDC) and male reproductive health have been observational, as interventions or randomization is not possible. Thus, only association not causation may be drawn. Also, for cross-sectional studies (which constitute the majority) reverse causation is a possibility, as the outcome may proceed the exposure. Participants with poor semen quality, earlier onset of puberty, or lower testosterone may have an unhealthier lifestyle in addition to the EDC exposure, and despite taking confounders into account in the data analysis, it is difficult to disentangle the adverse effect of one single exposure.

While it is challenging to study semen quality, therefore, many studies include men undergoing infertility treatment, as they are easier to recruit. Infertile men constitute a very heterogeneous population consisting of men with both impaired and normal fertility potential because of infertility due to female factors. Other investigations therefore include young healthy men or donors, it is, however, difficult to obtain a participation rate above 30% in such studies, and the participants may be healthier or have a greater concern about their fertility than non-participants. Many of the semen quality studies included few participants, and as both intra- and inter-individual variation in semen quality is large, this may explain the lack of associations found in these studies. Also, most studies are cross-sectional, and as semen takes approximately 90 days to mature it is difficult to assess causality. Often

questionnaires focus on EDC exposure, behavior, and lifestyle of the recent 4–6 weeks, and thus do not cover the entire timespan of the spermatogenesis process. In addition, due to the lack of intervention, the question whether a change of EDC exposure can actually restore or improve a reduced reproduction remains to be elucidated.

When studying timing of puberty onset, it is essential to perform a clinical examination. Alteration of hormone concentrations may be a biological responsiveness to protect the body system from EDC exposure. Follow-up is necessary to find if these alterations of hormone concentrations and disruption of puberty onset proceed to infertility or cause of other diseases, such as cancer in reproductive organs.

Many chemicals behave as endocrine disruptors. However, it is important to bear in mind that many chemicals have different exposure pathways, and endocrine disrupting may not be the most prominent. In addition, we are all exposed to these chemicals, and it is difficult to identify an unexposed group. Also, large spatial and temporal trends in exposure concentrations occurs, and it is not within the limits of this review to address these variations. Mixed exposure to various chemicals is also a considerable challenge in terms of delineating the possible health effects of chemical exposure. Humans are exposed to a complex mixture of EDCs, and when investigating the effect of one chemical, it may be another correlating co-exposure that actually does the harm, or the mixture of a number of chemicals, the so-called cocktail effect.

Lastly, genetic susceptibility may also modify the effects of EDCs on reproductive functions. The results from a Japanese cohort study suggest that maternal dioxin concentrations may differ due to variations in the aryl hydrocarbon receptor and polymorphisms in families of metabolizing enzymes such as cytochrome P450 [110]. A Russian cohort suggested that single nucleotide polymorphisms (SNPs) in the glucocorticoid receptor (GR/NR3C1) and estrogen receptor-α (ESR1) genes were significant modifiers of the association between peripubertal dioxin concentration and male pubertal onset [111]. Thus, it is also important to consider genetic factors in the relationship between environmental chemical exposure and reproductive health.

5.4 Conclusions

In this chapter, we review the literature on exposure to endocrine-disrupting chemicals, e.g., organochlorine compounds, perfluorinated alkylated substances, phthalates, and phenols on anogenital distance, reproductive hormones in childhood, onset of puberty, and semen quality focusing on prenatal or early exposures during vulnerable time points of development. Prenatal exposure to OCPs appears to lower children's testosterone concentrations and increase aromatase activity after birth. Prenatal exposure to dioxins and OCPs is consistently associated with delayed onset of puberty in both sexes, whereas PCBs accelerate the onset of puberty in boys. Generally, AGD appears to be a promising, easily obtainable marker of male repro-

ductive health. Maternal exposure to phthalates has consistently been associated with shorter AGD in male offspring, whereas childhood or adult phthalate exposure has been associated with lower reproductive hormone concentrations, changed the onset of puberty and semen quality. No consistent associations between PFAS or phenol exposure and AGD, reproductive hormones, puberty onset, or semen quality have been found; however, the number of studies is limited and further studies are urgently warranted. Future studies are suggested to be birth cohort studies focusing on the effect of exposures during vulnerable time windows during development, e.g., in utero, during early childhood and puberty. They should have the necessary size, include biological material, focus on multiple exposures, and have long-term follow-up with repeated clinical examinations.

References

1. WHO and UNEP. Global assessment of the state-of-the-science of endocrine disruptors. 2002.
2. WHO and UNEP. State of the science of endocrine disrupting chemicals-An assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme (UNEP) and WHO. 2012.
3. Gore AC, Chappell VA, Fenton SE, Flaws JA, Nadal A, Prins GS, et al. Executive summary to EDC-2: The Endocrine Society's second scientific statement on endocrine-disrupting chemicals. Endocr Rev. 2015;36(6):593–602.
4. Sharpe RM, Irvine DS. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? BMJ. 2004;328(7437):447–51.
5. Skakkebaek NE. Testicular dysgenesis syndrome: new epidemiological evidence. Int J Androl. 2004;27(4):189–91.
6. Juul A, Almstrup K, Andersson AM, Jensen TK, Jorgensen N, Main KM, et al. Possible fetal determinants of male infertility. Nat Rev Endocrinol. 2014;10(9):553–62.
7. Skakkebaek NE, Rajpert-De Meyts E, Buck Louis GM, Toppari J, Andersson AM, Eisenberg ML, et al. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol Rev. 2016;96(1):55–97.
8. Hotchkiss AK, Parks-Saldutti LG, Ostby JS, Lambright C, Furr J, Vandenbergh JG, et al. A mixture of the "antiandrogens" linuron and butyl benzyl phthalate alters sexual differentiation of the male rat in a cumulative fashion. Biol Reprod. 2004;71(6):1852–61.
9. Scott HM, Hutchison GR, Jobling MS, McKinnell C, Drake AJ, Sharpe RM. Relationship between androgen action in the "male programming window," fetal Sertoli cell number, and adult testis size in the rat. Endocrinology. 2008;149(10):5280–7.
10. Eisenberg ML, Hsieh MH, Walters RC, Krasnow R, Lipshultz LI. The relationship between anogenital distance, fatherhood, and fertility in adult men. PLoS One. 2011;6(5):e18973.
11. Mendiola J, Stahlhut RW, Jorgensen N, Liu F, Swan SH. Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. Environ Health Perspect. 2011;119(7):958–63.
12. Eisenberg ML, Jensen TK, Walters RC, Skakkebaek NE, Lipshultz LI. The relationship between anogenital distance and reproductive hormone levels in adult men. J Urol. 2012;187(2):594–8.
13. Thankamony A, Lek N, Carroll D, Williams M, Dunger DB, Acerini CL, et al. Anogenital distance and penile length in infants with hypospadias or cryptorchidism: comparison with normative data. Environ Health Perspect. 2014;122(2):207–11.

14. Convention S. Available from http://chm.pops.int/TheConvention/ThePOPs/ChemicalsProposedforListing/tabid/2510/Default.aspx.
15. Kanazawa A, Miyasita C, Okada E, Kobayashi S, Washino N, Sasaki S, et al. Blood persistent organochlorine pesticides in pregnant women in relation to physical and environmental variables in The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2012;426:73–82.
16. Warembourg C, Debost-Legrand A, Bonvallot N, Massart C, Garlantézec R, Monfort C, et al. Exposure of pregnant women to persistent organic pollutants and cord sex hormone levels. Hum Reprod. 2016;31(1):190–8.
17. Torres-Sanchez L, Zepeda M, Cebrián ME, Belkind-Gerson J, Garcia-Hernandez RM, Belkind-Valdovinos U, et al. Dichlorodiphenyldichloroethylene exposure during the first trimester of pregnancy alters the anal position in male infants. Ann N Y Acad Sci. 2008;1140(1):155–62.
18. Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer SP, Weber JP, Chapin RE, et al. *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, Mexico. Am J Epidemiol. 2007;165(9):1015–22.
19. Loreto-Gomez C, Farias P, Moreno-Macias H, Guzman C, Riojas-Rodriguez H. Prenatal exposure to persistent organic compounds and their association with anogenital distance in infants. Reprod Biomed Online. 2018;37(6):732–40.
20. Garcia-Villarino M, Riano-Galan I, Rodriguez-Dehli AC, Vizcaino E, Grimalt JO, Tardon A, et al. Prenatal exposure to persistent organic pollutants and anogenital distance in children at 18 months. Horm Res Paediatr. 2018;90(2):116–22.
21. Dalsager L, Christensen LE, Kongsholm MG, Kyhl HB, Nielsen F, Schoeters G, et al. Associations of maternal exposure to organophosphate and pyrethroid insecticides and the herbicide 2,4-D with birth outcomes and anogenital distance at 3 months in the Odense Child Cohort. Reprod Toxicol. 2018;76:53–62.
22. Lind DV, Priskorn L, Lassen TH, Nielsen F, Kyhl HB, Kristensen DM, et al. Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol. 2017;68:200–6.
23. Benjamin S, Masai E, Kamimura N, Takahashi K, Anderson RC, Faisal PA. Phthalates impact human health: epidemiological evidences and plausible mechanism of action. J Hazard Mater. 2017;340:360–83.
24. Katsikantami I, Sifakis S, Tzatzarakis MN, Vakonaki E, Kalantzi O-I, Tsatsakis AM, et al. A global assessment of phthalates burden and related links to health effects. Environ Int. 2016;97:212–36.
25. Ait Bamai Y, Araki A, Kawai T, Tsuboi T, Yoshioka E, Kanazawa A, et al. Comparisons of urinary phthalate metabolites and daily phthalate intakes among Japanese families. Int J Hyg Environ Health. 2015;218(5):461–70.
26. Fromme H, Bolte G, Koch HM, Angerer J, Boehmer S, Drexler H, et al. Occurrence and daily variation of phthalate metabolites in the urine of an adult population. Int J Hyg Environ Health. 2007;210(1):21–33.
27. Koch HM, Drexler H, Angerer J. Internal exposure of nursery-school children and their parents and teachers to di(2-ethylhexyl)phthalate (DEHP). Int J Hyg Environ Health. 2004;207(1):15–22.
28. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect. 2005;113(8):1056–61.
29. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res. 2008;108(2):177–84.
30. Cai H, Zheng W, Zheng P, Wang S, Tan H, He G, et al. Human urinary/seminal phthalates or their metabolite levels and semen quality: a meta-analysis. Environ Res. 2015;142:486–94.

31. Zarean M, Keikha M, Feizi A, Kazemitabaee M, Kelishadi R. The role of exposure to phthalates in variations of anogenital distance: a systematic review and meta-analysis. Environ Pollut. 2019;247:172–9.
32. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139–77.
33. Christiansen S, Axelstad M, Boberg J, Vinggaard AM, Pedersen GA, Hass U. Low-dose effects of bisphenol A on early sexual development in male and female rats. Reproduction. 2014;147(4):477–87.
34. Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E, et al. *In utero* exposure to bisphenol-A and anogenital distance of male offspring. Birth Defects Res Pt A. 2011;91(10):867–72.
35. Sun X, Li D, Liang H, Miao M, Song X, Wang Z, et al. Maternal exposure to bisphenol A and anogenital distance throughout infancy: a longitudinal study from Shanghai, China. Environ Int. 2018;121(Pt 1):269–75.
36. Liu C, Xu X, Zhang Y, Li W, Huo X. Associations between maternal phenolic exposure and cord sex hormones in male newborns. Hum Reprod. 2016;31(3):648–56.
37. Arbuckle TE, Agarwal A, MacPherson SH, Fraser WD, Sathyanarayana S, Ramsay T, et al. Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: The MIREC Study. Environ Int. 2018;120:572–83.
38. Mammadov E, Uncu M, Dalkan C. High prenatal exposure to bisphenol A reduces anogenital distance in healthy male newborns. J Clin Res Pediatr Endocrinol. 2018;10(1):25–9.
39. Hsu PC, Lai TJ, Guo NW, Lambert GH, Guo YL. Serum hormones in boys prenatally exposed to polychlorinated biphenyls and dibenzofurans. J Toxicol Environ Health A. 2005;68(17-18):1447–56.
40. Yang C-Y, Yu M-L, Guo H-R, Lai T-J, Hsu C-C, Lambert G, et al. The endocrine and reproductive function of the female Yucheng adolescents prenatally exposed to PCBs/PCDFs. Chemosphere. 2005;61(3):355–60.
41. Cao Y, Winneke G, Wilhelm M, Wittsiepe J, Lemm F, Fürst P, et al. Environmental exposure to dioxins and polychlorinated biphenyls reduce levels of gonadal hormones in newborns: results from The Duisburg Cohort Study. Int J Hyg Environ Health. 2008;211(1–2):30–9.
42. Araki A, Miyashita C, Mitsui T, Goudarzi H, Mizutani F, Chisaki Y, et al. Prenatal organochlorine pesticide exposure and the disruption of steroids and reproductive hormones in cord blood: The Hokkaido Study. Environ Int. 2018;110(Supplement C):1–13.
43. Eskenazi B, Rauch SA, Tenerelli R, Huen K, Holland NT, Lustig RH, et al. *In utero* and childhood DDT, DDE, PBDE and PCBs exposure and sex hormones in adolescent boys: The CHAMACOS Study. Int J Hyg Environ Health. 2017;220(2, Part B):364–72.
44. Vested A, Ramlau-Hansen CH, Olsen SF, Bonde JP, Stovring H, Kristensen SL, et al. *In utero* exposure to persistent organochlorine pollutants and reproductive health in the human male. Reproduction. 2014;148(6):635–46.
45. Toft G, Jonsson BA, Bonde JP, Norgaard-Pedersen B, Hougaard DM, Cohen A, et al. Perfluorooctane sulfonate concentrations in amniotic fluid, biomarkers of fetal Leydig cell function, and cryptorchidism and hypospadias in Danish boys (1980-1996). Environ Health Perspect. 2015;124(1):151–6.
46. Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, et al. The association of prenatal exposure to perfluorinated chemicals with glucocorticoid and androgenic hormones in cord blood samples: The Hokkaido Study. Environ Health Perspect. 2017;125(1):111–8.
47. Itoh S, Araki A, Mitsui T, Miyashita C, Goudarzi H, Sasaki S, et al. Association of perfluoroalkyl substances exposure *in utero* with reproductive hormone levels in cord blood in The Hokkaido Study on Environment and Children's Health. Environ Int. 2016;94:51–9.
48. Maisonet M, Näyhä S, Lawlor DA, Marcus M. Prenatal exposures to perfluoroalkyl acids and serum lipids at ages 7 and 15 in females. Environ Int. 2015;82:49–60.
49. Lin LC, Wang SL, Chang YC, Huang PC, Cheng JT, Su PH, et al. Associations between maternal phthalate exposure and cord sex hormones in human infants. Chemosphere. 2011;83(8):1192–9.

50. Araki A, Mitsui T, Miyashita C, Nakajima T, Naito H, Ito S, et al. Association between maternal exposure to di(2-ethylhexyl) phthalate and reproductive hormone levels in fetal blood: The Hokkaido Study on Environment and Children's Health. PLoS One. 2014;9(10):e109039.
51. Araki A, Mitsui T, Goudarzi H, Nakajima T, Miyashita C, Itoh S. Prenatal di(2-ethylhexyl) phthalate exposure and disruption of adrenal androgens and glucocorticoids levels in cord blood: The Hokkaido Study. Sci Total Environ. 2017;581-582:297–304.
52. Main KM, Mortensen GK, Kaleva MM, Boisen KA, Damgaard IN, Chellakooty M, et al. Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. Environ Health Perspect. 2006;114(2):270–6.
53. Wen HJ, Sie L, Su PH, Chuang CJ, Chen HY, Sun CW, et al. Prenatal and childhood exposure to phthalate diesters and sex steroid hormones in 2-, 5-, 8-, and 11-year-old children: a pilot study of the Taiwan Maternal and Infant Cohort Study. J Epidemiol. 2017;27(11):516–23.
54. Watkins DJ, Sanchez BN, Tellez-Rojo MM, Lee JM, Mercado-Garcia A, Blank-Goldenberg C, et al. Phthalate and bisphenol A exposure during *in utero* windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ Res. 2017;159:143–51.
55. Watkins DJ, Sanchez BN, Tellez-Rojo MM, Lee JM, Mercado-Garcia A, Blank-Goldenberg C, et al. Impact of phthalate and BPA exposure during *in utero* windows of susceptibility on reproductive hormones and sexual maturation in peripubertal males. Environ Health. 2017;16(1):69.
56. Fénichel P, Déchaux H, Harthe C, Gal J, Ferrari P, Pacini P, et al. Unconjugated bisphenol A cord blood levels in boys with descended or undescended testes. Hum Reprod. 2012;27(4):983–90.
57. Minatoya M, Sasaki S, Araki A, Miyashita C, Itoh S, Yamamoto J, et al. Cord blood bisphenol A levels and reproductive and thyroid hormone levels of neonates: The Hokkaido Study on Environment and Children's Health. Epidemiology. 2017;28:S3–9.
58. Kuijper EAM, Ket JCF, Caanen MR, Lambalk CB. Reproductive hormone concentrations in pregnancy and neonates: a systematic review. Reprod Biomed Online. 2013;27(1):33–63.
59. WHO. Endocrine disrupters and child health. Possible developmental early effects of endocrine disrupters on child health. Geneva: WHO; 2012.
60. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44(235):291–303.
61. Leijs MM, Koppe JG, Olie K, Aalderen WMCV, Voogt PD, Vulsma T, et al. Delayed initiation of breast development in girls with higher prenatal dioxin exposure; a longitudinal cohort study. Chemosphere. 2008;73(6):999–1004.
62. Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, et al. Prenatal exposure to persistent organochlorine pollutants and female reproductive function in young adulthood. Environ Int. 2016;92-93:366–72.
63. Namulanda G, Maisonet M, Taylor E, Flanders WD, Olson D, Sjodin A, et al. *In utero* exposure to organochlorine pesticides and early menarche in the Avon Longitudinal Study of Parents and Children. Environ Int. 2016;94:467–72.
64. Windham GC, Pinney SM, Voss RW, Sjodin A, Biro FM, Greenspan LC, et al. Brominated flame retardants and other persistent organohalogenated compounds in relation to timing of puberty in a longitudinal study of girls. Environ Health Perspect. 2015;123(10):1046–52.
65. Humblet O, Williams PL, Korrick SA, Sergeyev O, Emond C, Birnbaum LS, et al. Dioxin and polychlorinated biphenyl concentrations in mother's serum and the timing of pubertal onset in sons. Epidemiology. 2011;22(6):827–35.
66. Korrick SA, Lee MM, Williams PL, Sergeyev O, Burns JS, Patterson DG, et al. Dioxin exposure and age of pubertal onset among Russian boys. Environ Health Perspect. 2011;119(9):1339–44.
67. Lam T, Williams PL, Lee MM, Korrick SA, Birnbaum LS, Burns JS, et al. Prepubertal organochlorine pesticide concentrations and age of pubertal onset among Russian boys. Environ Int. 2014;73:135–42.

68. Burns JS, Lee MM, Williams PL, Korrick SA, Sergeyev O, Lam T, et al. Associations of peripubertal serum dioxin and polychlorinated biphenyl concentrations with pubertal timing among Russian boys. Environ Health Perspect. 2016;124(11):1801–7.
69. Den Hond E, Roels HA, Hoppenbrouwers K, Nawrot T, Thijs L, Vandermeulen C, et al. Sexual maturation in relation to polychlorinated aromatic hydrocarbons: Sharpe and Skakkebaek's hypothesis revisited. Environ Health Perspect. 2002;110(8):771–6.
70. Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, et al. Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Hum Reprod. 2013;28(12):3337–48.
71. Christensen KY, Maisonet M, Rubin C, Holmes A, Calafat AM, Kato K, et al. Exposure to polyfluoroalkyl chemicals during pregnancy is not associated with offspring age at menarche in a contemporary British cohort. Environ Int. 2011;37(1):129–35.
72. Lopez-Espinosa M-J, Fletcher T, Armstrong B, Genser B, Dhatariya K, Mondal D, et al. Association of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) with age of puberty among children living near a chemical plant. Environ Sci Technol. 2011;45(19):8160–6.
73. Su P-H, Chang C-K, Lin C-Y, Chen H-Y, Liao P-C, Hsiung CA, et al. Prenatal exposure to phthalate ester and pubertal development in a birth cohort in central Taiwan: a 12-year follow-up study. Environ Res. 2015;136(0):324–30.
74. Watkins DJ, Sánchez BN, Téllez-Rojo MM, Lee JM, Mercado-García A, Blank-Goldenberg C, et al. Phthalate and bisphenol A exposure during *in utero* windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ Res. 2017;159:143–51.
75. Watkins DJ, Sánchez BN, Téllez-Rojo MM, Lee JM, Mercado-García A, Blank-Goldenberg C, et al. Impact of phthalate and BPA exposure during *in utero* windows of susceptibility on reproductive hormones and sexual maturation in peripubertal males. Environ Health. 2017;16(1):69.
76. Wolff MS, Teitelbaum SL, Pinney SM, Windham G, Liao L, Biro F, et al. Investigation of relationships between urinary biomarkers of phytoestrogens, phthalates, and phenols and pubertal stages in girls. Environ Health Perspect. 2010;118(7):1039–46.
77. Wolff MS, Teitelbaum SL, McGovern K, Windham GC, Pinney SM, Galvez M, et al. Phthalate exposure and pubertal development in a longitudinal study of US girls. Hum Reprod. 2014;29(7):1558–66.
78. Wolff MS, Pajak A, Pinney SM, Windham GC, Galvez M, Rybak M, et al. Associations of urinary phthalate and phenol biomarkers with menarche in a multiethnic cohort of young girls. Reprod Toxicol. 2017;67:56–64.
79. Mieritz MG, Frederiksen H, Sørensen K, Aksglaede L, Mouritsen A, Hagen CP, et al. Urinary phthalate excretion in 555 healthy Danish boys with and without pubertal gynaecomastia. Int J Androl. 2012;35(3):227–35.
80. Frederiksen H, Sørensen K, Mouritsen A, Aksglaede L, Hagen CP, Petersen JH, et al. High urinary phthalate concentration associated with delayed pubarche in girls. Int J Androl. 2012;35(3):216–26.
81. Mouritsen A, Frederiksen H, Sørensen K, Aksglaede L, Hagen C, Skakkebaek NE, et al. Urinary phthalates from 168 girls and boys measured twice a year during a 5-year period: associations with adrenal androgen levels and puberty. J Clin Endocrinol Metab. 2013;98(9):3755–64.
82. Zhang Y, Cao Y, Shi H, Jiang X, Zhao Y, Fang X, et al. Could exposure to phthalates speed up or delay pubertal onset and development? A 1.5-year follow-up of a school-based population. Environ Int. 2015;83:41–9.
83. Leonardi A, Cofini M, Rigante D, Lucchetti L, Cipolla C, Penta L, et al. The effect of bisphenol A on puberty: a critical review of the medical literature. Int J Environ Res Public Health. 2017;14(9):E1044.

84. Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, et al. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet. 1998;352(9135):1172–7.
85. Jensen TK, Jacobsen R, Christensen K, Nielsen NC, Bostofte E. Good semen quality and life expectancy: a cohort study of 43,277 men. Am J Epidemiol. 2009;170(5):559–65.
86. Eisenberg ML, Li S, Behr B, Cullen MR, Galusha D, Lamb DJ, et al. Semen quality, infertility and mortality in the USA. Hum Reprod. 2014;29(7):1567–74.
87. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ. 1992;305(6854):609–13.
88. Levine H, Jorgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update. 2017;23(6):646–59.
89. Vested A, Giwercman A, Bonde JP, Toft G. Persistent organic pollutants and male reproductive health. Asian J Androl. 2014;16(1):71–80.
90. Petersen MS, Halling J, Jorgensen N, Nielsen F, Grandjean P, Jensen TK, et al. Reproductive function in a population of young faroese men with elevated exposure to polychlorinated biphenyls (PCBs) and perfluorinated alkylate substances (PFAS). Int J Environ Res Public Health. 2018;15(9):E1880.
91. Petersen MS, Halling J, Weihe P, Jensen TK, Grandjean P, Nielsen F, et al. Spermatogenic capacity in fertile men with elevated exposure to polychlorinated biphenyls. Environ Res. 2015;138:345–51.
92. Aneck-Hahn NH, Schulenburg GW, Bornman MS, Farias P, de Jager C. Impaired semen quality associated with environmental DDT exposure in young men living in a malaria area in the Limpopo Province, South Africa. J Androl. 2007;28(3):423–34.
93. De Jager C, Farias P, Barraza-Villarreal A, Avila MH, Ayotte P, Dewailly E, et al. Reduced seminal parameters associated with environmental DDT exposure and p,p′-DDE concentrations in men in Chiapas, Mexico: a cross-sectional study. J Androl. 2006;27(1):16–27.
94. Mocarelli P, Gerthoux PM, Needham LL, Patterson DG Jr, Limonta G, Falbo R, et al. Perinatal exposure to low doses of dioxin can permanently impair human semen quality. Environ Health Perspect. 2011;119(5):713–8.
95. Wang YX, You L, Zeng Q, Sun Y, Huang YH, Wang C, et al. Phthalate exposure and human semen quality: results from an infertility clinic in China. Environ Res. 2015;142:1–9.
96. Wang YX, Zeng Q, Sun Y, Yang P, Wang P, Li J, et al. Semen phthalate metabolites, semen quality parameters and serum reproductive hormones: a cross-sectional study in China. Environ Pollut. 2016;211:173–82.
97. Wang YX, Zhou B, Chen YJ, Liu C, Huang LL, Liao JQ, et al. Thyroid function, phthalate exposure and semen quality: exploring associations and mediation effects in reproductive-aged men. Environ Int. 2018;116:278–85.
98. Radke EG, Braun JM, Meeker JD, Cooper GS. Phthalate exposure and male reproductive outcomes: a systematic review of the human epidemiological evidence. Environ Int. 2018;121(Pt 1):764–93.
99. Axelsson J, Rylander L, Rignell-Hydbom A, Lindh CH, Jonsson BA, Giwercman A. Prenatal phthalate exposure and reproductive function in young men. Environ Res. 2015;138:264–70.
100. Hart RJ, Frederiksen H, Doherty DA, Keelan JA, Skakkebaek NE, Minaee NS, et al. The possible impact of antenatal exposure to ubiquitous phthalates upon male reproductive function at 20 years of age. Front Endocrinol. 2018;9:288.
101. Bach CC, Vested A, Jørgensen KT, Bonde JPE, Henriksen TB, Toft G. Perfluoroalkyl and polyfluoroalkyl substances and measures of human fertility: a systematic review. Crit Rev Toxicol. 2016;46(9):735–55.
102. Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE, Jorgensen N. Do perfluoroalkyl compounds impair human semen quality? Environ Health Perspect. 2009;117(6):923–7.

103. Toft G, Jonsson BA, Lindh CH, Giwercman A, Spano M, Heederik D, et al. Exposure to perfluorinated compounds and human semen quality in Arctic and European populations. Hum Reprod. 2012;27(8):2532–40.
104. Louis GM, Chen Z, Schisterman EF, Kim S, Sweeney AM, Sundaram R, et al. Perfluorochemicals and human semen quality: the LIFE study. Environ Health Perspect. 2015;123(1):57–63.
105. Den Hond E, Tournaye H, De Sutter P, Ombelet W, Baeyens W, Covaci A, et al. Human exposure to endocrine disrupting chemicals and fertility: a case-control study in male subfertility patients. Environ Int. 2015;84:154–60.
106. Vested A, Ramlau-Hansen CH, Olsen SF, Bonde JP, Kristensen SL, Halldorsson TI, et al. Associations of *in utero* exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environ Health Perspect. 2013;121(4):453–8.
107. Minguez-Alarcon L, Hauser R, Gaskins AJ. Effects of bisphenol A on male and couple reproductive health: a review. Fertil Steril. 2016;106(4):864–70.
108. Li S, Dai J, Zhang L, Zhang J, Zhang Z, Chen B. An association of elevated serum prolactin with phthalate exposure in adult men. Biomed Environ Sci. 2011;24(1):31–9.
109. Lassen TH, Jensen TK, Petersen JH, Joensen UN, Main KM, Skakkebæk NE, Juul A, Jørgensen N, Andersson AM. Elevated urinary bisphenol A excretion associated with higher serum testosterone, estradiol and LH and lower percentage progressive motile sperm in young men. Environ Health Perspect. 2013;122(5):478–84.
110. Kobayashi S, Sata F, Sasaki S, Ban S, Miyashita C, Okada E, et al. Genetic association of aromatic hydrocarbon receptor (AHR) and cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) polymorphisms with dioxin blood concentrations among pregnant Japanese women. Toxicol Lett. 2013;219(3):269–78.
111. Humblet O, Korrick SA, Williams PL, Sergeyev O, Emond C, Birnbaum LS, et al. Genetic modification of the association between peripubertal dioxin exposure and pubertal onset in a cohort of Russian boys. Environ Health Perspect. 2013;121(1):111–7.
112. Miyashita C, Araki A, Mitsui T, Itoh S, Goudarzi H, Sasaki S, et al. Sex-related differences in the associations between maternal dioxin-like compounds and reproductive and steroid hormones in cord blood: The Hokkaido Study. Environ Int. 2018;117:175–85.
113. Grandjean P, Grønlund C, Kjær IM, Jensen TK, Sørensen N, Andersson A-M, et al. Reproductive hormone profile and pubertal development in 14-year-old boys prenatally exposed to polychlorinated biphenyls. Reprod Toxicol. 2012;34(4):498–503.
114. Kolatorova L, Vitku J, Hampl R, Adamcova K, Skodova T, Simkova M, et al. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. Environ Res. 2018;163:115–22.

Chapter 6
Thyroid Hormone System and Development

Sachiko Itoh

Abstract In recent years, many studies have been published regarding thyroid hormones (THs)-disrupting effects of environmental chemicals. THs play a crucial role in normal fetal growth and maturation. Even before the fetal thyroid gland matures and starts to secrete THs, THs are detected in the fetal cerebral cortex, thereby confirming that fetuses completely rely on the maternal TH supply by the end of the first trimester. Therefore, exposure of pregnant women and their fetus to environmental chemicals that can interfere with THs has been of special concern. This chapter covers mainly recent findings on the epidemiological studies which investigated the associations between prenatal exposure to environmental chemicals and neonatal TH concentrations. In addition, possible mechanisms of thyroid-disrupting action by each chemical are drawn such as competitive bindings to TH transport proteins, interference of TH regulation, synthesis, and metabolism. Further, we emphasize a need for longitudinal studies to more completely assess whether the effects of chemical exposure on TH level, particularly during sensitive developmental windows, such as in fetal life and early infancy, are permanent.

Keywords Thyroid hormones · Environmental chemicals · Endocrine disrupters · Prenatal exposure

6.1 Importance of Thyroid Hormones

Thyroid hormones (THs) play a critical role in numerous physiological processes, including the regulation of metabolism, growth and bone remodeling, and development of the nervous system. The main THs, namely thyroxine (T4) and

S. Itoh (✉)
Center for Environmental Health and Sciences, Hokkaido University, Sapporo, Japan
e-mail: vzbghjn@den.hokudai.ac.jp

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_6

3,5,3′-triiodothyronine (T3), are produced by the thyroid gland and widely recognized as essential for fetal development, especially in terms of central nervous system and brain maturation, throughout gestation [1, 2].

The fetal thyroid gland matures by week 11–12 and starts to secrete THs by week 16–18 [3]. However, by week 12, T4 and T3 are already detected in the fetal cerebral cortex, thereby confirming that fetuses rely on maternal TH supply completely by the end of the first trimester [4, 5]. Even after the maturation of the fetal thyroid gland, maternal THs with essential iodine supplies are transferred to the fetus throughout pregnancy [6].

Previous studies have found that even a mild decrease in the level of maternal THs in early pregnancy is related to a lower intelligence quotient (IQ) among neonates [7, 8]. Moreover, a Danish cohort study reported that maternal hyperthyroidism diagnosed within 2 years of a child's birth increased the risk of attention deficit hyperactivity disorder (ADHD), whereas hypothyroidism increased the risk of autism spectrum disorder (ASD) [9]. Higher maternal free T4 (FT4) levels in the early stages of gestation are also associated with a lower birth weight and an increased risk of small-for-gestational age [10]. Therefore, abnormal levels of maternal THs during pregnancy may impair fetal development. Globally, for the detection of thyroid dysfunction and initiation of treatment in the early stages, TH screening programs have been established for pregnant women and their neonates by using blood spot thyroid-stimulating hormone (TSH) or T4 tests or both.

6.2 Regulation of Thyroid Hormone Levels

Circulatory TH levels are strictly maintained within a very narrow range by a negative feedback regulation system in the hypothalamus-pituitary-thyroid (HPT) axis (Fig. 6.1). Thyrotropin-releasing hormone (TRH) is released from the hypothalamus and reaches the TSH-producing cells of the anterior pituitary. The released TSH then binds to the receptor in the thyroid gland, initiating TH synthesis; subsequently, TH is released predominantly as T4—a prohormone—and at low concentrations as an active hormone T3. THs have to be transported into cells for the exertion of their own effects. T4 has to be converted to T3 by deiodinating enzymes, since only T3 can bind to the TH receptor (TR). There are three types of deiodinases, namely type 1 (D1), type 2 (D2), and type 3 (D3).

More than 98% of THs in circulation bind to TH transporter proteins thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin [11]. In humans, TBG is the main transporter protein. TTR is not a major T4 transporter, and its binding capacity is weaker than that of TBG; however, it is considered to be the most important T4 carrier protein in fetuses because it can transfer placenta and cross the blood–brain barrier [12]. TTR is also reported to determine the FT4 levels in the brain's extracellular compartment.

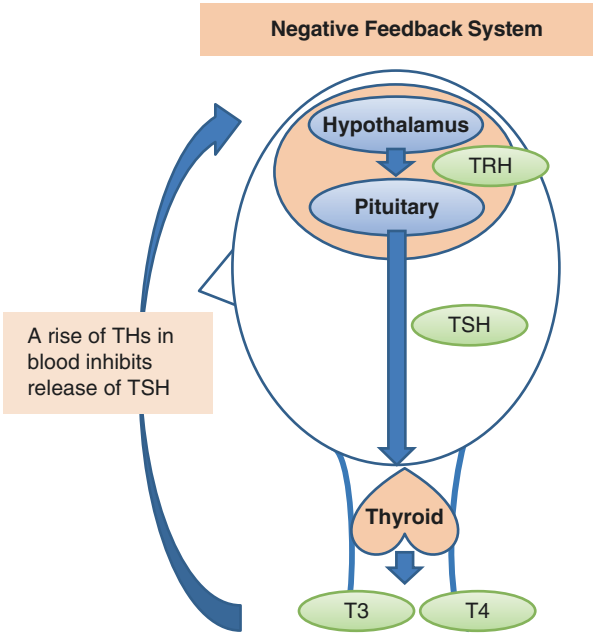


Fig. 6.1 Regulation of thyroid hormone concentrations by negative feedback system. Thyroid hormone (TH) concentrations are strictly controlled via feedback loop system. Thyrotropin-releasing hormone (TRH) is released from hypothalamus and reaches the TSH-producing cells of the anterior pituitary. Released thyroid-stimulating hormone (TSH) stimulates TH synthesis and subsequently T3 and T4 are released in the circulation. Once a rise of THs in blood is detected, it exerts a negative feedback at the level of the hypothalamus and pituitary, leading the inhibition of TSH releasing

6.3 Possible Mechanisms of Thyroid Disruption by Environmental Chemicals

In recent years, many studies have reported on the thyroid-disrupting effects of environmental chemicals. These chemicals are highly persistent and bio-accumulative, and humans and wildlife are constantly exposed to them. Of special concern is the adverse effect of environmental chemicals on pregnant women and their fetus and infants, as thyroid disruption during pregnancy and the early development stage may cause serious neurological dysfunction.

Some in vitro and animal studies have revealed that environmental chemical compounds, such as polychlorinated biphenyl (PCB), dioxin, and perfluoroalkyl substances (PFASs), affect TH levels. There are several possible thyroid-disrupting mechanisms behind this (Fig. 6.2). First, endocrine-disrupting chemicals (EDCs) may directly affect the thyroid gland through the disruption of TH production, interference with receptors and iodine uptake, and inhibition of thyroid peroxidase or

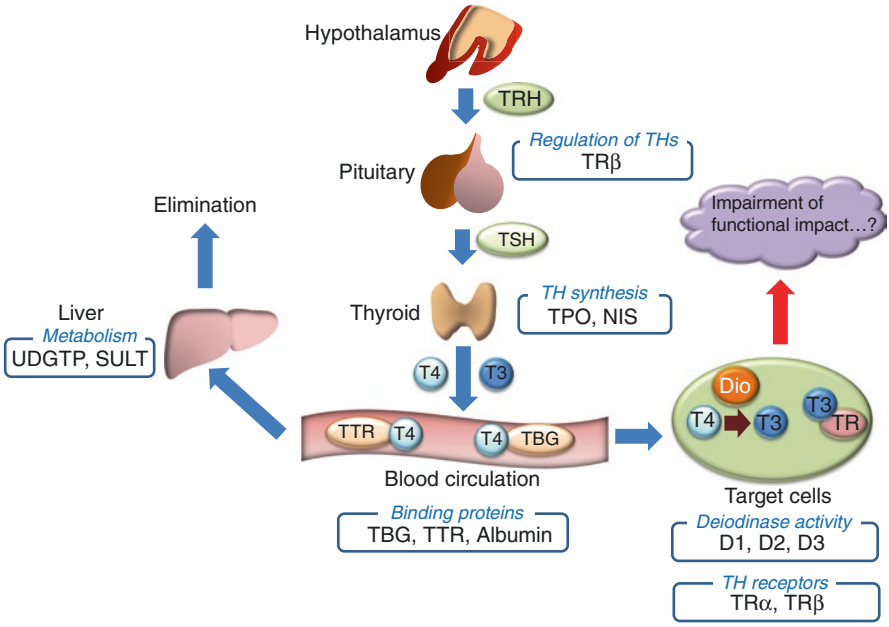


Fig. 6.2 Possible mechanisms of disruption of thyroid hormone homeostasis by EDCs. The pathway of thyroid hormone release, transport, and metabolism. Hypothalamus-pituitary-thyroid (HPT) axis and potential target sites may be disrupted by endocrine disrupting chemicals (EDCs) (Framed actions). *Dio* deiodinase, *NIS* sodium iodide symporter, *SULT* sulfotransferase, *TBG* thyroid biding globulin, *TH* thyroid hormone, *TPO* thyroid peroxidase, *TRH* thyroid releasing hormone, *TSH* thyroid-stimulating hormone, *TTR* transthyretin, *T4* thyroxine, *T3* triiodothyronine, *UDGTP* uridine diphosphate glucuronosyltransferase

sodium iodide symporter (NIS) activity. For example, perchlorate and thiocyanate are known as NIS inhibitors which block iodide uptake into the thyroid gland. Phthalates also change the activity of the NIS, leading to changes in iodide uptake. Second, altered TH levels may be caused by increased biliary excretion. THs are metabolized in the liver by phase two enzymes, namely, sulfotransferase (sulfation) and uridine diphosphate glucuronosyltransferase (conjugation). These enzymes are stimulated by PCBs, dioxin, and Bisphenol A (BPA), leading to the faster excretion of THs. Third, EDCs have a structure that is similar to that of T4. These compounds, mainly POPs such as PCBs, PFASs and PBDEs, competitively bind to TTR instead of T4 itself, leading to a decrease in TH concentrations, while TSH levels may also be disrupted as compensation. As mentioned above, TTR is not among the main TH transporters in humans. However, its binding to EDCs facilitates the transportation of EDCs across the placenta to the fetal compartment. In addition, EDCs can also reach targeted organs such as the brain. THs are transported across the targeted cells of targeted organs. In order to exert their biological effects, THs bind to the TR of the nucleus. Some EDCs reportedly interact with TR and disrupt TR expression by affecting the TR genes.

6.4 Thyroid Hormones and Environmental Chemicals

6.4.1 Polychlorinated Biphenyls and Dioxin

PCBs and dioxins (polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans) are persistent organic pollutants that have the potential to seriously harm human health. PCBs are among the oldest environmental chemicals and were produced, as early as 1881, in Germany. PCBs were widely used in the industrial production of plasticizers, lubricants, electrical equipment, as well as hydraulic and heat transfer fluids. Their manufacture was banned in the mid-1970s due to growing evidence on their toxic effects. However, the general population continues to be exposed to them thorough old products and contaminated food because of their long half-lives and strong bioaccumulation through the food chain. In addition, it is of great concern that they have the ability to cross the placenta, leading to fetal exposure. In particular, the adverse effects of PCBs on neurodevelopment and growth in infancy and early childhood have been reported on. A previous study conducted in the USA showed that the children of women who consumed PCB-contaminated fish from Lake Michigan showed lower IQ scores and behavioral deficits [13]. Subsequently, prenatal exposure to PCBs has been linked with reduced cognitive function [14], mental and motor deficits [15], and increased risk of ADHD and ASD development [16, 17]. Based on these and other studies, the neurodevelopmental impairment associated with PCB exposure in utero has been suspected to disrupt TH homeostasis, which regulates growth and development.

The presumed mechanism of toxicity of PCBs and dioxins is mainly the displacement of T4 with TTR. Numerous experimental publications have confirmed the association of PCB or dioxin exposure with reduced TH levels, especially T4 [18–20]. Moreover, experimental animal studies have presented substantial evidence that in utero exposure to the hydroxylated metabolites of PCB, namely OH-PCBs, affects TH concentrations in offspring [21, 22]. In fact, OH-PCBs are reported to possess a much higher binding affinity to TTR than T4, and their parent compounds, i.e., PCBs [23–25]. The binding of OH-PCBs to TTR is also a matter of great concern because it could promote the distribution of OH-PCBs to the placenta and even the brain. Recent evidence indicates that OH-PCBs may be more toxic than PCBs, although the toxicological effects of these compounds have not been entirely revealed. Additionally, OH-PCBs may inhibit TH sulfation and the D1 activities that convert T4 to T3 or the inactive metabolites (reverse T3), leading to the alteration of T4 levels in circulation [26]. Exposure to dioxins induces UDP-glucuronosyltransferase (UDPGT) activity and the increased excretion of T4 into bile [27]. Dioxins also activate the aryl hydrocarbon receptor (AhR) and thereby interfere with the UDPGTs, as well as PCBs via the constitutive androstane receptor (CAR) and pregnane × receptor (PXR) [28, 29]. Dioxins reportedly decrease the strength of dose-level liver deiodinase activity [30].

As shown in Table 6.1, epidemiological studies have reported that prenatal exposure to PCBs may reduce the TH levels in neonates and infants [31–34] or increase

Table 6.1 Overview of previous reports on POPs exposure and neonatal or infant thyroid hormone parameters (sorted by published year)

Author/year of publish	Year of research	Country	Sample size	Specimen for exposure assessment	Specimen for outcome assessment	PCBs-Dioxins			PFASs								PBDE
						PCDD/PCDF	PCB	OH-PCB	PFPeA	PFHxS	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFTrDA	
Preston et al. 2018 [70]		USA	480	Maternal blood	Heel pick					T4↓	T4↓	T4↓	–				
Itoh et al. 2018 [51]	2002–2005	Japan	222	Maternal blood	Heel pick (day 4)			FT4↑									
Baba et al. 2018 [38]	2002–2006	Japan	385	Maternal blood	Heel pick (day 5)	FT4↑	FT4↑										
Soechitram et al. 2017 [39]	1998–2000	The Netherlands	100	Maternal blood	Cord blood Infant blood (at 3 and 18 months)		Cord blood T3↑, rT3↓	3 months blood T4↑, T3↑, TSH↓ 18 months blood T4↑, T3↑, TSH↓, T4S↑									
Tsai et al. 2017 [71]	2004–2005	Taiwan	118	Cord blood	Cord blood						TSH↑ T4↓	–	–		–		
Berg et al. 2016 [34]	2007–2009	Norway	391	Maternal blood	Heel pick		T3↓			–	TSH↑	–	–	T3↓, FT3↓	–		
Ding et al. 2017 [85]	2010–2011	China	123	Cord blood	Cord blood												TT4↑
Shah-Kulkarni et al. 2016 [74]	2006–2010	Korea	279	Cord blood	Cord blood				T4↑, T3↓	T3↑	–	–	TSH↓		–	–	
Kato et al. 2016 [73]	2002–2005	Japan	392	Maternal blood	Heel pick (day 4)						TSH↓	–					

Leonetti et al. 2016 [86]	2010–2011	USA	95	Placenta	Placenta												T3↑, rT3↓
Kim et al. 2015 [87]		Korea	104	Maternal blood	Cord blood Heel pick (2–7 day after birth)		–										–
				Cord blood	Cord blood Heel pick (2–7 day after birth)		–										Cord blood TSH↑ Heel pick TSH↑
Vuong et al. 2015 [84]	2003–2006	USA	389	Maternal blood	Cord blood												FT3↓
de Cock et al. 2014 [40]	2011–2013	The Netherlands	83	Cord blood	Heel pick		–				–	T4↑					
Wang et al. 2014 [72]	2005–2006	Taiwan	116	Maternal blood	Cord blood					–	–	–	TT3↓, TT4↓	–	–		
Hisada et al. 2013 [41]	2009–2010	Japan	79	Maternal blood	Heel pick (day 5)		–	TSH↑									
Abdelouahab et al. 2013 [50]	2007–2008	Canada	380	Maternal blood	Cord blood		–										FT4↓
Kim et al. 2011 [69]	2008–2009	South Korea	44	Maternal blood	Cord blood						T3↓	–				T3↓T4↓	
				Cord blood	Cord blood						–	–				–	
Chevrier et al. 2011 [88]	1999–2000	USA	289	Maternal blood	Heel pick (after 24 h)												–

(continued)

Table 6.1 (continued)

Author/year of publish	Year of research	Country	Sample size	Specimen for exposure assessment	Specimen for outcome assessment	PCBs-Dioxins			PFASs								PBDE
						PCDD/PCDF	PCB	OH-PCB	PFPeA	PFHxS	PFOS	PFOA	PFNA	PFDA	PFUnDA	PFTrDA	
Lin et al. 2011 [83]	2007–2008	Taiwan	54	Cord blood	Cord blood												T3↓, FT3↓, T4/T3 ratio↑
Lopez-Espinosa et al. 2010 [42]	2004–2006	Spain	453	Cord blood	Heel pick (>day 2)		–										
Darnerud et al. 2010 [33]	1996–1999	Sweden	150	Maternal blood	Infant blood (at 3 weeks/3 months)	–	TT3↓										
Dallaire et al. 2009 [43]	1995–2001	Canada	130	Maternal bloodCord blood	Cord blood		–	–									
Kim 2009	2007	Korea	108	Cord blood	Cord blood												–
Alvarez-Pedrerol et al. 2008 [36]	1997–1998	Spain	27	Cord blood	Infant blood (day 3)		TSH↑										
Wilhelm et al. 2008 [44]	2000–2002	Germany	84	Maternal blood	Cord blood	–	–										
Dallaire et al. 2008 [45]	1993–1997	Canada	2 cohorts 410 and 260	Cord blood	Cord blood		–										

Herbstman et al. 2008 [32]	2004–2005	USA	289	Cord blood	Cord blood Heel pick (day 2)		Cord blood TT4↓, FT4↓ Heel pick TT4↓										Cord blood TT4↓, FT4↓
Maervoet et al. 2007 [31]	2002–2004	Belgium	198	Cord blood	Cord blood	FT3↓ FT4↓	FT3↓ FT4↓										
Chevrier et al. 2007 [35]	1999–2000	USA	285	Maternal blood	Heel pick (after 25 h)		TSH↑										
Otake et al. 2007 [46]	2005	Japan	23	Placental tissue	Heel pick		–	FT4↑									
Wang et al. 2005 [37]	2000–2001	Taiwan	118	Placenta	Cord blood	–	FT4 × TSH↓										
Takser et al. 2005 [47]		Canada	92	Maternal blood Cord blood	Cord blood		–										
Ribas-Fitó 2003 [48]	1997–1999	Spain	70	Cord blood	Infant blood (day 3)		–										
Steuerwald et al. 2000 [49]	1994–1995	Denmark	182	Maternal blood	Cord blood		–										

↑/↓: arrows are indicated as follows: ↓ inversely associated ($p < 0.05$); ↑ positively associated ($p < 0.05$), and [–] no significant association. Blanks indicate not measured in the study

the TSH levels [35, 36]. This indicates that the TSH level is increased as a compensation by decreased peripheral TH levels. Low TH and/or high TSH levels are suggestive of hypothyroidism, as observed in experimental studies. Previous studies conducted in Taiwan have shown that high PCB levels in the placenta were associated with low FT4 × TSH levels in cord blood. This result indicates that PCBs may disrupt the peripheral hormonal balance by affecting the negative feedback system for the maintenance of TH levels [37]. However, some studies found increased TH levels [38, 39] or no significant association between PCBs and THs [40–50].

Regarding prenatal OH-PCB exposure, four of five previous studies demonstrated that it led to TH and TSH disruption [39, 41, 46, 51]. While it is noteworthy that Hisada et al. [41] and Otake et al. [46] observed significant associations between OH-PCBs and THs or TSH, they found no significant association in terms of PCBs. These findings suggest that OH-PCBs may have a stronger effect on THs than PCBs. Furthermore, Soechitram et al. [39] explored the associations of OH-PCBs in maternal blood during pregnancy with the TH levels in their children at three time points, i.e., at birth (cord blood), at 3 months, and 18 months, after birth; they reported that maternal PCB exposure was associated with cord blood TH levels, while maternal OH-PCB exposure was related to TH levels at 3 and 18 months after birth. This indicates that the effect of OH-PCB on THs lasts longer than that of PCBs. Additionally, the results of the aforementioned studies indicate that OH-PCBs have greater toxicity than PCBs. A Canadian study showed no such association among the Inuit [43], even though the exposure level of these people was higher than that observed in other studies, owing to a high consumption of PCB-containing fish. This may be explained by the fact that fish also contain a high amount of iodine. Iodine is an element required for the production of THs, and sufficient intake may prevent thyroid dysfunction.

Regarding dioxins, studies in Japan and Belgium have shown associations between dioxin exposure and neonatal TH levels, although the directions of the results varied [31, 38]. Possible reasons for the discrepancies in the results may be differences in the specimens used for the assessment of dioxin and TH levels. The Belgian study used cord blood for the assessment of both dioxins and THs, while the Japanese study measured the levels of dioxin in maternal blood during pregnancy and THs after birth. Another reason may be the difference in iodine intake via dietary habit. Three previous studies that examined the same association did not demonstrate the presence of any significant association [33, 44, 52].

Overall, epidemiological studies have shown that prenatal exposure to PCBs and dioxins interfere with the TH levels in neonates even after the production of those chemicals was banned. However, there is still inconsistency in the literature in terms of the relationship between PCB and dioxin exposure and TH levels, despite the firm associations between PCB and dioxin exposure and adverse neurodevelopment effects. Further studies are needed to assess whether impaired neurodevelopment is fully explained by disrupted THs in utero and in the early stages of life.

6.4.2 *Perfluoroalkyl Substances*

PFASs are widely used in industrial products such as surfactants, Teflon, carpets, fire-fighting foams, photographic films, and cosmetics due to their unique surfactant properties. They are very stable and therefore extremely persistent and are commonly accumulated in the environment. Human exposure to PFASs mainly occurs orally via the intake of contaminated food, releases from food packaging, drinking water, and dust [53]. As perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are the most commonly detected PFASs in the environment and in humans, their presence in human blood has been reported globally [54–57]. In 2009, PFOS was added to Annex B of the Stockholm Convention on Persistent Organic Pollutants, and PFOA was also proposed to be listed in the Stockholm Convention in 2018. Although PFOS and PFOA are being voluntarily phased out by several industries, they are still present in older products and have long elimination half-lives in human serum (PFOS: 5.3 years, PFOA: 3.8 years) [58]. Recently, other PFASs have replaced PFOS and PFOA, such as perfluorononanoic acid and perfluorodecanoic acid [59]. All these PFASs can cross the placental barrier and be transferred from mother to fetus in humans [57, 60, 61]. Therefore, significant concerns have been raised regarding the adverse effects of in utero exposure to PFASs on the fetus. PFASs reportedly change the expression of synaptophysin and tau proteins in the cerebral cortex and hippocampus, which are essential for normal brain development [62]. Hence, prenatal PFAS exposure may impair infants' neurodevelopment through decreasing TH levels or by other means.

In vitro studies have suggested two possible mechanisms of TH disruption by PFASs; one is the competitive binding to TTR as well as other persistent organic pollutants (POPs) [63] and another is the upregulation of hepatic glucuronidation enzymes and deiodinase in the thyroid gland [64]. Several animal studies have found altered levels of TH after in utero exposure to PFASs. Prenatal exposure to PFOS reduced the T4 levels in rat pups as well as pregnant dams, and postnatal PFOS exposure decreased the T4 levels among rat pups [65–68].

In humans, several studies have examined the effect of PFASs on neonate or infant TH levels (Table 6.1). The effects of PFOS and PFOA exposure have been extensively explored, as much as in nine previous studies. Kim et al. investigated the association of PFASs in maternal blood sera and cord blood with neonatal TH levels in cord blood among participants in South Korea (n = 44) [69]. They reported a negative correlation between maternal PFOS and fetal total T3 and between maternal perfluorotridecanoic acid (PFTrDA) and total T3 and T4 in fetuses; moreover, maternal PFOA was positively correlated with fetal TSH. In addition, other studies have shown the inverse associations of PFAS exposure with TH levels in cord blood or a heel-prick blood test [34, 70–72]. These results of reduced TH levels are consistent with those observed in animal studies. One Japanese study found an inverse association of PFOS with TSH, while no association was observed with THs [73]. As TSH levels are more sensitive than T4 or T3 levels due to strict regulation by the negative feedback system, it may be reasonable to consider that altered TSH levels

are a result of affected THs in circulation. However, TH levels may also increase as a result of PFAS exposure [40, 74]. A Dutch study reported that a high level of PFOA in cord blood was associated with increased total T4 levels in girls, as measured during heel-prick blood draws (n = 83) [40]. In a Korean study, short-chain PFASs such as perfluoro-*n*-pentanoic acid (PFPeA) and perfluorohexanoic acid were associated with increased T3 and T4 levels in girls, while PFPeA was associated with reduced T3 levels in boys [74].

In summary, the effect of PFAS toxicity on TH disruption has generally been established in epidemiological studies. However, there are still issues to be investigated, particularly in terms of which PFASs are likely to have the strongest effect on neonatal THs, as not all PFASs pass through the placenta to fetuses at an equal rate.

6.4.3 Polybrominated Flame Retardants

Polybrominated diphenyl ethers (PBDEs) are synthetic chemicals widely used as flame retardants due to their high flash point. PBDEs are a group of POPs known for their properties such as bioaccumulation, lipophilicity, and persistence [75]. The structures of PBDEs are similar to those of PCBs; therefore, the adverse health impacts of PBDEs are a matter of concern.

Experimental studies have proposed some mechanisms by which PBDEs may interfere with TH levels. PBDEs bind to TH transporters, TBG and TTR, as well as other POPs, leading to a reduction in the T4 level [76, 77]. The binding of PBDEs to TRs is considered to lead to the suppression of T3 levels by the inhibition of T3 binding to TRs [78, 79]. In addition, decabromodiphenyl ether exposure in zebrafish parents downregulates the gene expression of TRs, with a decrease in T4 level, in offspring [80]. PBDEs can also alter thyroid deiodinase activity and upregulate the expression of clearance enzymes, leading to decreased hormone levels in circulation [81, 82]

Human studies have confirmed the effects of PBDE on TH disruption, although the directions of the associations are not consistent (Table 6.1). Three studies in Canada, Taiwan, and the USA reported that PBDE exposure in utero was associated with reduced levels of TH, T3, and T4 [32, 50, 83, 84]. One Chinese study found a positive association between PBDE levels and T3 in cord blood [85]. Leonetti et al. used PBDE and TH levels in placenta and observed associations between PBDE exposure and increased T3 levels in girls and decreased rT3 levels in boys [86]. A Korean study found associations between cord blood PBDE and increased TSH levels in cord blood and a heel-prick blood draw without alterations in the T3 or T4 levels [87], which are considered subclinical symptoms of hypothyroidism. On the other hand, a study in the USA reported no significant association between PBDE presence in maternal blood and neonatal TSH [88]. A Korean study showed no significant association between PBDEs and THs in cord blood [89].

PBDEs can be metabolized in human liver tissue and be converted to hydroxylated PBDEs (OH-PBDEs) [90]. OH-PBDEs have structures that are more similar to those of THs than PBDEs; however, few studies have investigated the potent adverse effects of OH-PBDEs on THs. OH-PBDEs should also be explored for the clarification of the underlying mechanisms of endocrine disruption.

6.4.4 Phthalates

Phthalates are a group of chemicals widely used as plastic emollients and additives in various consumer and industrial products. Their half-lives of elimination are relatively short compared to those of POPs; therefore, they do not accumulate in the body. However, they are included in daily necessaries, leading to continuous exposure.

In addition to competitive binding to TTR like POPs [91], the underlying mechanisms behind thyroid dysregulation by phthalate exposure have been examined in animal and in vitro studies. Previous studies have demonstrated histopathological changes in the thyroid of rats in association with bis(2-ethylhexyl) phthalate (DEHP) exposure [92, 93]. Some phthalates increase the activity of the human NIS promoter [94] and modulate the transcriptional activity of NIS, leading to changes in iodide uptake in rat thyroid cells [95]. Phthalate exposure also changes the transcription of the genes in the HPT axis and expression of TR genes [96, 97]. Furthermore, a recent study showed that DEHP downregulated the expression of the TRH receptor in the hypothalamus, upregulated the protein and mRNA levels of the TRH receptor in the pituitary gland, and downregulated the mRNA expression of the TSH receptor in the thyroid [98]. DEHP can affect TH levels through not only biosynthesis and biotransportation but also biotransformation [99].

Although some possible mechanisms of TH disruption as a result of phthalate exposure have been reported on, few epidemiological studies have examined the association between prenatal exposure to phthalates and neonatal or infant TH levels (Table 6.2). Huang et al. investigated the relationships between phthalates in maternal urine at three visits during pregnancy and TH levels in cord blood in a Taiwanese population. They reported that molar sum of urinary di-*n*-butyl phthalate (ΣDBPm) metabolites in the second trimester was associated with increased T3 and FT4 levels in cord blood, and mono-*n*-butyl phthalate exposure in the second trimester was associated with increased cord T3 levels [100]. The presence of phthalates in maternal urine in the first or third trimesters was not associated with cord TH levels. Another Taiwanese study found that the presence of methylbenzylpiperazine in maternal urine during the third trimester was inversely associated with cord TSH levels [101]. However, three studies conducted in Japan, the Netherlands, and China found no associations between prenatal phthalate exposure and TH levels in cord blood or a heel-prick blood test [40, 102, 103].

To draw firm conclusions, further studies should focus on the temporal profile of phthalate exposure during gestation.

6.4.5 *Bisphenols*

Bisphenol A (BPA) is an organic synthetic compound widely used in the production of plastic products such as food and drink containers, medical devices, and thermal paper receipts. BPA has been detected in amniotic fluid, cord blood, and placental tissue, indicating in utero exposure. Although the use of BPA in baby bottles and plastic food containers has been restricted in the USA and European countries, BPA exposure is still ubiquitous. Furthermore, a number of structural BPA analogues such as bisphenol S (BPS), bisphenol F (BPF), and bisphenol B (BPB) have replaced BPA.

As shown in Table 6.2, the effect of in utero exposure to BPA on neonatal TH levels is not consistent. One study in the USA observed an inverse association between BPA in maternal urine and boys' TSH levels on a heel-prick blood draw [104]. Another USA study also found an association between maternal urine BPA and decreased TSH levels in cord blood; however, this association was observed only in girls [105]. Minatoya et al. reported no association between BPA in cord blood and neonatal TH levels as estimated by a heel-prick blood test [106].

Mechanistic studies have indicated several pathways by which BPA could interfere with TH levels. BPA has been reported to have the ability to bind to the transport proteins of THs; however, its potency is weaker than that of environmental chemicals such as PCBs and PBDEs [107, 108]. A possible mechanism of BPA in the disruption of THs is TR binding. BPA shows T3-TR-mediated antagonistic effects by the suppression of transcriptional activity [109]. Another study suggested that BPA acts as an antagonist on the beta-TR [110]. Exposure to BPA and its analogues may change the thyroid-related gene expression of cells in the pituitary and thyroid glands [111–113]. BPA was shown to increase the activities of biotransformation enzymes such as glutathione S-transferase and UDPGT [114]. Few studies have assessed the effects of BPS, BPF, and BPB on thyroid function. Further study should focus on not only BPA but also its analogues.

6.5 Future Directions of Research on Thyroid Hormones

Several epidemiological studies have shown the thyroid-disrupting effects of various chemicals, through experimental animal and in vitro studies. However, the results of the studies are inconsistent. This may be attributed to the different periods in which biological samples were obtained for both chemical exposure and TH concentrations. As for exposure assessment, many studies used maternal blood or cord blood. Maternal blood volume increases during pregnancy; therefore, the blood obtained at different periods during pregnancy may have different concentrations of chemicals. Moreover, different chemicals pass from mothers to fetuses across the placenta at varying rates. Therefore, the chemical concentrations in maternal blood are likely to differentially represent the concentrations in cord blood or fetuses.

Table 6.2 Overview of previous reports on phthalates and BPA exposure and neonatal or infant thyroid hormone parameters (sorted by published year)

Author/ year of publish	Year of research	Country	Sample size	Specimen for exposure assessment	Specimen for outcome assessment	Phthalates											BPA
						ΣDBPm	MMP	MEP	MiBP	MnBP	MEHP	MEHHP	MEOHP	MECPP	MCMHP	MBzP	
Huang et al. 2018 [100]	2013–2014	Taiwan	98	Maternal urine	Cord blood	T3, FT4↑	–	–	–	T3↑	–	–	–	–	–		
Minatoya et al. 2016 [102]	2002–2005	Japan	328	Maternal blood	Heel pick (day 4)						–						
Yao et al. 2016 [103]		China	2521	Maternal urine	Cord blood		–	–	–		–	–	–			–	
Kuo et al. 2015 [101]	2009–2010	Taiwan	148	Maternal urine	Cord blood		–	–	–	–	–	–	–			TSH↓	
de Cock et al. 2014 [40]	2011–2013	The Netherlands	83	Cord blood	Heel pick							–	–	–			
Minatoya et al. 2017 [106]	2002–2004	Japan	283	Cord blood	Heel pick (day 3)												–
Romano et al. 2015 [105]	2003–2006	USA	249	Maternal urine (16 and 26 weeks gestation)	Cord blood												TSH↓
Chevrier et al. 2013 [104]	1999–2000	USA	364	Maternal urine	Heel pick												TSH↓

↑/↓: arrows are indicated as follows: ↓ inversely associated ($p < 0.05$); ↑ positively associated ($p < 0.05$), and [–] no significant association. Blanks indicate not measured in the study

As for neonatal THs, the levels in cord blood are affected by many factors such as mode of delivery, gestational age, duration and other additional factors. Since heel-prick blood is usually obtained 2–5 days after birth, the concentrations of THs are much more stable than those in cord blood. The various results obtained for neonatal THs need careful interpretation, taking the aforementioned different conditions into account.

In the future, there is a need for longitudinal studies to assess whether the effects of chemical exposure interference on TH levels during sensitive developmental windows such as in fetal life and early infancy are permanent. In particular, neurological development should be investigated, as the fetal stage is critical for the development of the central nervous system. Further study should also focus on understanding the effects of exposure to various environmental chemical mixtures, as exposure to various chemicals is ubiquitous in daily life. As iodide intake is essential, those with insufficiency or pre-existing thyroid disease may be more vulnerable.

Acknowledgements This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18gk0110032.

References

1. Joffe RT, Sokolov ST. Thyroid hormones, the brain, and affective disorders. Crit Rev Neurobiol. 1994;8(1-2):45–63.
2. Neale DM, Cootauco AC, Burrow G. Thyroid disease in pregnancy. Clin Perinatol. 2007;34(4):543–57.
3. Obregon MJ, Calvo RM, Del Rey FE, de Escobar GM. Ontogenesis of thyroid function and interactions with maternal function. Endocr Dev. 2007;10:86–98.
4. Calvo RM, Jauniaux E, Gulbis B, Asuncion M, Gervy C, Contempre B, et al. Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. J Clin Endocrinol Metab. 2002;87(4):1768–77.
5. Kester MH, Martinez de Mena R, Obregon MJ, Marinkovic D, Howatson A, Visser TJ, et al. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. J Clin Endocrinol Metab. 2004;89(7):3117–28.
6. de Escobar GM, Obregon MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract Res Clin Endocrinol Metab. 2004;18(2):225–48.
7. LaFranchi SH, Austin J. How should we be treating children with congenital hypothyroidism? J Pediatr Endocrinol Metab. 2007;20(5):559–78.
8. Gyamfi C, Wapner RJ, D'Alton ME. Thyroid dysfunction in pregnancy: the basic science and clinical evidence surrounding the controversy in management. Obstet Gynecol. 2009;113(3):702–7.
9. Andersen SL, Laurberg P, Wu CS, Olsen J. Attention deficit hyperactivity disorder and autism spectrum disorder in children born to mothers with thyroid dysfunction: A Danish Nationwide Cohort Study. BJOG. 2014;121(11):1365–74.
10. Medici M, de Rijke YB, Peeters RP, Visser W, de Muinck Keizer-Schrama SM, Jaddoe VV, et al. Maternal early pregnancy and newborn thyroid hormone parameters: The Generation R Study. J Clin Endocrinol Metab. 2012;97(2):646–52.

11. Kodavanti PR, Curras-Collazo MC. Neuroendocrine actions of organohalogens: thyroid hormones, arginine vasopressin, and neuroplasticity. Front Neuroendocrinol. 2010;31(4):479–96.
12. Schreiber G, Southwell BR, Richardson SJ. Hormone delivery systems to the brain-transthyretin. Exp Clin Endocrinol Diabetes. 1995;103(2):75–80.
13. Jacobson JL, Jacobson SW. Dose-response in perinatal exposure to polychlorinated biphenyls (PCBs): the Michigan and North Carolina Cohort Studies. Toxicol Ind Health. 1996;12(3-4):435–45.
14. Schantz SL, Widholm JJ, Rice DC. Effects of PCB exposure on neuropsychological function in children. Environ Health Perspect. 2003;111(3):357–576.
15. Koopman-Esseboom C, Weisglas-Kuperus N, de Ridder MA, Van der Paauw CG, Tuinstra LG, Sauer PJ. Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infants' mental and psychomotor development. Pediatrics. 1996;97(5):700–6.
16. Sagiv SK, Thurston SW, Bellinger DC, Tolbert PE, Altshul LM, Korrick SA. Prenatal organochlorine exposure and behaviors associated with attention deficit hyperactivity disorder in school-aged children. Am J Epidemiol. 2010;171(5):593–601.
17. Lyall K, Croen LA, Sjodin A, Yoshida CK, Zerbo O, Kharrazi M, et al. Polychlorinated biphenyl and organochlorine pesticide concentrations in maternal mid-pregnancy serum samples: association with autism spectrum disorder and intellectual disability. Environ Health Perspect. 2017;125(3):474–80.
18. Crofton KM. Thyroid disrupting chemicals: mechanisms and mixtures. Int J Androl. 2008;31(2):209–23.
19. Crofton KM, Craft ES, Hedge JM, Gennings C, Simmons JE, Carchman RA, et al. Thyroid-hormone-disrupting chemicals: evidence for dose-dependent additivity or synergism. Environ Health Perspect. 2005;113(11):1549–54.
20. van der Plas SA, Lutkeschipholt I, Spenkelink B, Brouwer A. Effects of subchronic exposure to complex mixtures of dioxin-like and non-dioxin-like polyhalogenated aromatic compounds on thyroid hormone and vitamin A levels in female Sprague-Dawley rats. Toxicol Sci. 2001;59(1):92–100.
21. Meerts IA, Assink Y, Cenijn PH, Van Den Berg JH, Weijers BM, Bergman A, et al. Placental transfer of a hydroxylated polychlorinated biphenyl and effects on fetal and maternal thyroid hormone homeostasis in the rat. Toxicol Sci. 2002;68(2):361–71.
22. Gabrielsen KM, Villanger GD, Lie E, Karimi M, Lydersen C, Kovacs KM, et al. Levels and patterns of hydroxylated polychlorinated biphenyls (OH-PCBs) and their associations with thyroid hormones in hooded seal (*Cystophora cristata*) mother-pup pairs. Aquat Toxicol. 2011;105(3-4):482–91.
23. Brouwer A, Morse DC, Lans MC, Schuur AG, Murk AJ, Klasson-Wehler E, et al. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol Ind Health. 1998;14(1-2):59–84.
24. Lans MC, Klasson-Wehler E, Willemsen M, Meussen E, Safe S, Brouwer A. Structure-dependent, competitive interaction of hydroxy-polychlorobiphenyls, -dibenzo-p-dioxins and -dibenzofurans with human transthyretin. Chem Biol Interact. 1993;88(1):7–21.
25. Meerts IA, van Zanden JJ, Luijks EA, van Leeuwen-Bol I, Marsh G, Jakobsson E, et al. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicol Sci. 2000;56(1):95–104.
26. Schuur AG, Legger FF, van Meeteren ME, Moonen MJ, van Leeuwen-Bol I, Bergman A, et al. In vitro inhibition of thyroid hormone sulfation by hydroxylated metabolites of halogenated aromatic hydrocarbons. Chem Res Toxicol. 1998;11(9):1075–81.
27. Nishimura N, Yonemoto J, Miyabara Y, Sato M, Tohyama C. Rat thyroid hyperplasia induced by gestational and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. Endocrinology. 2003;144(5):2075–83.
28. White SS, Birnbaum LS. An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. J Environ Sci Health C. 2009;27(4):197–211.

29. Hernandez JP, Mota LC, Baldwin WS. Activation of CAR and PXR by dietary, environmental and occupational chemicals alters drug metabolism, intermediary metabolism, and cell proliferation. Curr Pharmacogenomics Pers Med. 2009;7(2):81–105.
30. Viluksela M, Raasmaja A, Lebofsky M, Stahl BU, Rozman KK. Tissue-specific effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the activity of 5′-deiodinases I and II in rats. Toxicol Lett. 2004;147(2):133–42.
31. Maervoet J, Vermeir G, Covaci A, Van Larebeke N, Koppen G, Schoeters G, et al. Association of thyroid hormone concentrations with levels of organochlorine compounds in cord blood of neonates. Environ Health Perspect. 2007;115(12):1780–6.
32. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, et al. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. Environ Health Perspect. 2008;116(10):1376–82.
33. Darnerud PO, Lignell S, Glynn A, Aune M, Tornkvist A, Stridsberg M. POP levels in breast milk and maternal serum and thyroid hormone levels in mother-child pairs from Uppsala, Sweden. Environ Int. 2010;36(2):180–7.
34. Berg V, Nost TH, Pettersen RD, Hansen S, Veyhe AS, Jorde R, et al. Persistent organic pollutants and the association with maternal and infant thyroid homeostasis: a multipollutant assessment. Environ Health Perspect. 2016;125(1):127–33.
35. Chevrier J, Eskenazi B, Bradman A, Fenster L, Barr DB. Associations between prenatal exposure to polychlorinated biphenyls and neonatal thyroid-stimulating hormone levels in a Mexican-American population, Salinas Valley, California. Environ Health Perspect. 2007;115(10):1490–6.
36. Alvarez-Pedrerol M, Ribas-Fito N, Torrent M, Carrizo D, Garcia-Esteban R, Grimalt JO, et al. Thyroid disruption at birth due to prenatal exposure to beta-hexachlorocyclohexane. Environ Int. 2008;34(6):737–40.
37. Wang S-L, Su P-H, Jong S-B, Guo YL, Chou W-L, Päpke O. In utero exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. Environ Health Perspect. 2005;113(11):1645–50.
38. Baba T, Ito S, Yuasa M, Yoshioka E, Miyashita C, Araki A, et al. Association of prenatal exposure to PCDD/Fs and PCBs with maternal and infant thyroid hormones: The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2018;615:1239–46.
39. Soechitram SD, Berghuis SA, Visser TJ, Sauer PJ. Polychlorinated biphenyl exposure and deiodinase activity in young infants. Sci Total Environ. 2017;574:1117–24.
40. de Cock M, de Boer MR, Lamoree M, Legler J, van de Bor M. Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants – a Dutch prospective cohort study. Environ Health. 2014;13:106.
41. Hisada A, Shimodaira K, Okai T, Watanabe K, Takemori H, Takasuga T, et al. Associations between levels of hydroxylated PCBs and PCBs in serum of pregnant women and blood thyroid hormone levels and body size of neonates. Int J Hyg Environ Health. 2013;217(4-5):546–53.
42. Lopez-Espinosa MJ, Vizcaino E, Murcia M, Fuentes V, Garcia AM, Rebagliato M, et al. Prenatal exposure to organochlorine compounds and neonatal thyroid stimulating hormone levels. J Expo Sci Environ Epidemiol. 2010;20(7):579–88.
43. Dallaire R, Muckle G, Dewailly E, Jacobson SW, Jacobson JL, Sandanger TM, et al. Thyroid hormone levels of pregnant inuit women and their infants exposed to environmental contaminants. Environ Health Perspect. 2009;117(6):1014–20.
44. Wilhelm M, Wittsiepe J, Lemm F, Ranft U, Kramer U, Furst P, et al. The Duisburg Birth Cohort Study: influence of the prenatal exposure to PCDD/Fs and dioxin-like PCBs on thyroid hormone status in newborns and neurodevelopment of infants until the age of 24 months. Mutat Res. 2008;659(1-2):83–92.
45. Dallaire R, Dewailly E, Ayotte P, Muckle G, Laliberte C, Bruneau S. Effects of prenatal exposure to organochlorines on thyroid hormone status in newborns from two remote coastal regions in Quebec, Canada. Environ Res. 2008;108(3):387–92.

46. Otake T, Yoshinaga J, Enomoto T, Matsuda M, Wakimoto T, Ikegami M, et al. Thyroid hormone status of newborns in relation to in utero exposure to PCBs and hydroxylated PCB metabolites. Environ Res. 2007;105(2):240–6.
47. Takser L, Mergler D, Baldwin M, de Grosbois S, Smargiassi A, Lafond J. Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury. Environ Health Perspect. 2005;113(8):1039–45.
48. Ribas-Fito N, Sala M, Cardo E, Mazon C, De Muga ME, Verdu A, et al. Organochlorine compounds and concentrations of thyroid stimulating hormone in newborns. Occup Environ Med. 2003;60(4):301–3.
49. Steuerwald U, Weihe P, Jorgensen PJ, Bjerve K, Brock J, Heinzow B, et al. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. J Pediatr. 2000;136(5):599–605.
50. Abdelouahab N, Langlois MF, Lavoie L, Corbin F, Pasquier JC, Takser L. Maternal and cord-blood thyroid hormone levels and exposure to polybrominated diphenyl ethers and polychlorinated biphenyls during early pregnancy. Am J Epidemiol. 2013;178(5):701–13.
51. Itoh S, Baba T, Yuasa M, Miyashita C, Kobayashi S, Araki A, et al. Association of maternal serum concentration of hydroxylated polychlorinated biphenyls with maternal and neonatal thyroid hormones: The Hokkaido Birth Cohort Study. Environ Res. 2018;167:583–90.
52. Wang SL, Su PH, Jong SB, Guo YL, Chou WL, Papke O. In utero exposure to dioxins and polychlorinated biphenyls and its relations to thyroid function and growth hormone in newborns. Environ Health Perspect. 2005;113(11):1645–50.
53. Fromme H, Tittlemier SA, Volkel W, Wilhelm M, Twardella D. Perfluorinated compounds--exposure assessment for the general population in Western countries. Int J Hyg Environ Health. 2009;212(3):239–70.
54. Butenhoff JL, Olsen GW, Pfahles-Hutchens A. The applicability of biomonitoring data for perfluorooctanesulfonate to the environmental public health continuum. Environ Health Perspect. 2006;114(11):1776–82.
55. Calafat AM, Kuklenyik Z, Reidy JA, Caudill SP, Tully JS, Needham LL. Serum concentrations of 11 polyfluoroalkyl compounds in the U.S. population: data from the national health and nutrition examination survey (NHANES). Environ Sci Technol. 2007;41(7):2237–42.
56. Harada K, Koizumi A, Saito N, Inoue K, Yoshinaga T, Date C, et al. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate contamination in human serum in Japan. Chemosphere. 2007;66(2):293–301.
57. Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health. 2007;80(7):643–8.
58. Olsen GW, Zobel LR. Assessment of lipid, hepatic, and thyroid parameters with serum perfluorooctanoate (PFOA) concentrations in fluorochemical production workers. Int Arch Occup Environ Health. 2007;81(2):231–46.
59. Okada E, Kashino I, Matsuura H, Sasaki S, Miyashita C, Yamamoto J, et al. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003-2011. Environ Int. 2013;60:89–96.
60. Gutzkow KB, Haug LS, Thomsen C, Sabaredzovic A, Becher G, Brunborg G. Placental transfer of perfluorinated compounds is selective–a Norwegian Mother and Child Sub-cohort Study. Int J Hyg Environ Health. 2012;215(2):216–9.
61. Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect. 2004;112(11):1204–7.
62. Lee I, Viberg H. A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain. Neurotoxicology. 2013;37:190–6.

63. Weiss JM, Andersson PL, Lamoree MH, Leonards PE, van Leeuwen SP, Hamers T. Competitive binding of poly- and perfluorinated compounds to the thyroid hormone transport protein transthyretin. Toxicol Sci. 2009;109(2):206–16.
64. Yu WG, Liu W, Jin YH. Effects of perfluorooctane sulfonate on rat thyroid hormone biosynthesis and metabolism. Environ Toxicol Chem. 2009;28(5):990–6.
65. Lau C, Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Stanton ME, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci. 2003;74(2):382–92.
66. Luebker DJ, York RG, Hansen KJ, Moore JA, Butenhoff JL. Neonatal mortality from in utero exposure to perfluorooctanesulfonate (PFOS) in Sprague-Dawley rats: dose-response, and biochemical and pharamacokinetic parameters. Toxicology. 2005;215(1-2):149–69.
67. Yu WG, Liu W, Jin YH, Liu XH, Wang FQ, Liu L, et al. Prenatal and postnatal impact of perfluorooctane sulfonate (PFOS) on rat development: a cross-foster study on chemical burden and thyroid hormone system. Environ Sci Technol. 2009;43(21):8416–22.
68. Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, et al. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse I: maternal and prenatal evaluations. Toxicol Sci. 2003;74(2):369–81.
69. Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, et al. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol. 2011;45(17):7465–72.
70. Preston EV, Webster TF, Oken E, Claus Henn B, McClean MD, Rifas-Shiman SL, et al. Maternal plasma per- and polyfluoroalkyl substance concentrations in early pregnancy and maternal and neonatal thyroid function in a prospective birth cohort: project viva (USA). Environ Health Perspect. 2018;126(2):027013.
71. Tsai MS, Lin CC, Chen MH, Hsieh WS, Chen PC. Perfluoroalkyl substances and thyroid hormones in cord blood. Environ Pollut. 2017;222:543–8.
72. Wang Y, Rogan WJ, Chen PC, Lien GW, Chen HY, Tseng YC, et al. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan Maternal and Infant Cohort Study. Environ Health Perspect. 2014;122(5):529–34.
73. Kato S, Itoh S, Yuasa M, Baba T, Miyashita C, Sasaki S, et al. Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environ Health Prev Med. 2016;21(5):334–44.
74. Shah-Kulkarni S, Kim BM, Hong YC, Kim HS, Kwon EJ, Park H, et al. Prenatal exposure to perfluorinated compounds affects thyroid hormone levels in newborn girls. Environ Int. 2016;94:607–13.
75. Hooper K, McDonald TA. The PBDEs: an emerging environmental challenge and another reason for breast-milk monitoring programs. Environ Health Perspect. 2000;108(5):387–92.
76. Ren XM, Guo LH. Assessment of the binding of hydroxylated polybrominated diphenyl ethers to thyroid hormone transport proteins using a site-specific fluorescence probe. Environ Sci Technol. 2012;46(8):4633–40.
77. Cao J, Lin Y, Guo LH, Zhang AQ, Wei Y, Yang Y. Structure-based investigation on the binding interaction of hydroxylated polybrominated diphenyl ethers with thyroxine transport proteins. Toxicology. 2010;277(1-3):20–8.
78. Kitamura S, Kato T, Iida M, Jinno N, Suzuki T, Ohta S, et al. Anti-thyroid hormonal activity of tetrabromobisphenol A, a flame retardant, and related compounds: affinity to the mammalian thyroid hormone receptor, and effect on tadpole metamorphosis. Life Sci. 2005;76(14):1589–601.
79. Fini JB, Le Mevel S, Turque N, Palmier K, Zalko D, Cravedi JP, et al. An in vivo multiwell-based fluorescent screen for monitoring vertebrate thyroid hormone disruption. Environ Sci Technol. 2007;41(16):5908–14.
80. Han Z, Li Y, Zhang S, Song N, Xu H, Dang Y, et al. Prenatal transfer of decabromodiphenyl ether (BDE-209) results in disruption of the thyroid system and developmental toxicity in zebrafish offspring. Aquat Toxicol. 2017;190:46–52.

81. Szabo DT, Richardson VM, Ross DG, Diliberto JJ, Kodavanti PR, Birnbaum LS. Effects of perinatal PBDE exposure on hepatic phase I, phase II, phase III, and deiodinase 1 gene expression involved in thyroid hormone metabolism in male rat pups. Toxicol Sci. 2009;107(1):27–39.
82. Hoffman K, Sosa JA, Stapleton HM. Do flame retardant chemicals increase the risk for thyroid dysregulation and cancer? Curr Opin Oncol. 2017;29(1):7–13.
83. Lin SM, Chen FA, Huang YF, Hsing LL, Chen LL, Wu LS, et al. Negative associations between PBDE levels and thyroid hormones in cord blood. Int J Hyg Environ Health. 2011;214(2):115–20.
84. Vuong AM, Webster GM, Romano ME, Braun JM, Zoeller RT, Hoofnagle AN, et al. Maternal polybrominated diphenyl ether (PBDE) exposure and thyroid hormones in maternal and cord sera: the home study, Cincinnati, USA. Environ Health Perspect. 2015;123(10):1079–85.
85. Ding G, Yu J, Chen L, Wang C, Zhou Y, Hu Y, et al. Polybrominated diphenyl ethers (PBDEs) and thyroid hormones in cord blood. Environ Pollut. 2017;229:489–95.
86. Leonetti C, Butt CM, Hoffman K, Hammel SC, Miranda ML, Stapleton HM. Brominated flame retardants in placental tissues: associations with infant sex and thyroid hormone endpoints. Environ Health. 2016;15(1):113.
87. Kim S, Park J, Kim HJ, Lee JJ, Choi G, Choi S, et al. Association between several persistent organic pollutants and thyroid hormone levels in cord blood serum and bloodspot of the Newborn Infants of Korea. PLoS One. 2015;10(5):e0125213.
88. Chevrier J, Harley KG, Bradman A, Sjodin A, Eskenazi B. Prenatal exposure to polybrominated diphenyl ether flame retardants and neonatal thyroid-stimulating hormone levels in the CHAMACOS study. Am J Epidemiol. 2011;174(10):1166–74.
89. Kim TH, Lee YJ, Lee E, Patra N, Lee J, Kwack SJ, et al. Exposure assessment of polybrominated diphenyl ethers (PBDE) in umbilical cord blood of Korean infants. J Toxicol Environ Health Part A. 2009;72(21–22):1318–26.
90. Lupton SJ, McGarrigle BP, Olson JR, Wood TD, Aga DS. Human liver microsome-mediated metabolism of brominated diphenyl ethers 47, 99, and 153 and identification of their major metabolites. Chem Res Toxicol. 2009;22(11):1802–9.
91. Ishihara A, Nishiyama N, Sugiyama S, Yamauchi K. The effect of endocrine disrupting chemicals on thyroid hormone binding to Japanese quail transthyretin and thyroid hormone receptor. Gen Comp Endocrinol. 2003;134(1):36–43.
92. Poon R, Lecavalier P, Mueller R, Valli VE, Procter BG, Chu I. Subchronic oral toxicity of di-n-octyl phthalate and di(2-Ethylhexyl) phthalate in the rat. Food Chem Toxicol. 1997;35(2):225–39.
93. Howarth JA, Price SC, Dobrota M, Kentish PA, Hinton RH. Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. Toxicol Lett. 2001;121(1):35–43.
94. Breous E, Wenzel A, Loos U. The promoter of the human sodium/iodide symporter responds to certain phthalate plasticisers. Mol Cell Endocrinol. 2005;244(1-2):75–8.
95. Wenzel A, Franz C, Breous E, Loos U. Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. Mol Cell Endocrinol. 2005;244(1-2):63–71.
96. Zhai W, Huang Z, Chen L, Feng C, Li B, Li T. Thyroid endocrine disruption in zebrafish larvae after exposure to mono-(2-ethylhexyl) phthalate (MEHP). PLoS One. 2014;9(3):e92465.
97. Sugiyama S, Shimada N, Miyoshi H, Yamauchi K. Detection of thyroid system-disrupting chemicals using in vitro and in vivo screening assays in Xenopus laevis. Toxicol Sci. 2005;88(2):367–74.
98. Sun D, Zhou L, Wang S, Liu T, Zhu J, Jia Y, et al. Effect of Di-(2-ethylhexyl) phthalate on the hypothalamus-pituitary-thyroid axis in adolescent rat. Endocr J. 2018;65(3):261–8.
99. Liu C, Zhao L, Wei L, Li L. DEHP reduces thyroid hormones via interacting with hormone synthesis-related proteins, deiodinases, transthyretin, receptors, and hepatic enzymes in rats. Environ Sci Pollut Res Int. 2015;22(16):12711–9.
100. Huang HB, Kuo PL, Chang JW, Jaakkola JJK, Liao KW, Huang PC. Longitudinal assessment of prenatal phthalate exposure on serum and cord thyroid hormones homeostasis during pregnancy - Tainan birth cohort study (TBCS). Sci Total Environ. 2018;619-620:1058–65.

101. Kuo FC, Su SW, Wu CF, Huang MC, Shiea J, Chen BH, et al. Relationship of urinary phthalate metabolites with serum thyroid hormones in pregnant women and their newborns: a prospective birth cohort in Taiwan. PLoS One. 2015;10(6):e0123884.
102. Minatoya M, Naka Jima S, Sasaki S, Araki A, Miyashita C, Ikeno T, et al. Effects of prenatal phthalate exposure on thyroid hormone levels, mental and psychomotor development of infants: The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2016;565:1037–43.
103. Yao HY, Han Y, Gao H, Huang K, Ge X, Xu YY, et al. Maternal phthalate exposure during the first trimester and serum thyroid hormones in pregnant women and their newborns. Chemosphere. 2016;157:42–8.
104. Chevrier J, Gunier RB, Bradman A, Holland NT, Calafat AM, Eskenazi B, et al. Maternal urinary bisphenol a during pregnancy and maternal and neonatal thyroid function in the CHAMACOS study. Environ Health Perspect. 2013;121(1):138–44.
105. Romano ME, Webster GM, Vuong AM, Thomas Zoeller R, Chen A, Hoofnagle AN, et al. Gestational urinary bisphenol A and maternal and newborn thyroid hormone concentrations: the HOME Study. Environ Res. 2015;138:453–60.
106. Minatoya M, Araki A, Nakajima S, Sasaki S, Miyashita C, Yamazaki K, et al. Cord blood BPA level and child neurodevelopment and behavioral problems: The Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2017;607-608:351–6.
107. Kudo Y, Yamauchi K. In vitro and in vivo analysis of the thyroid disrupting activities of phenolic and phenol compounds in Xenopus laevis. Toxicol Sci. 2005;84(1):29–37.
108. Marchesini GR, Meimaridou A, Haasnoot W, Meulenberg E, Albertus F, Mizuguchi M, et al. Biosensor discovery of thyroxine transport disrupting chemicals. Toxicol Appl Pharmacol. 2008;232(1):150–60.
109. Moriyama K, Tagami T, Akamizu T, Usui T, Saijo M, Kanamoto N, et al. Thyroid hormone action is disrupted by bisphenol A as an antagonist. J Clin Endocrinol Metab. 2002;87(11):5185–90.
110. Zoeller RT, Bansal R, Parris C. Bisphenol-A, an environmental contaminant that acts as a thyroid hormone receptor antagonist in vitro, increases serum thyroxine, and alters RC3/neurogranin expression in the developing rat brain. Endocrinology. 2005;146(2):607–12.
111. Seiwa C, Nakahara J, Komiyama T, Katsu Y, Iguchi T, Asou H. Bisphenol A exerts thyroid-hormone-like effects on mouse oligodendrocyte precursor cells. Neuroendocrinology. 2004;80(1):21–30.
112. Nakamura K, Itoh K, Yaoi T, Fujiwara Y, Sugimoto T, Fushiki S. Murine neocortical histogenesis is perturbed by prenatal exposure to low doses of Bisphenol A. J Neurosci Res. 2006;84(6):1197–205.
113. Lee S, Kim C, Youn H, Choi K. Thyroid hormone disrupting potentials of bisphenol A and its analogues - in vitro comparison study employing rat pituitary (GH3) and thyroid follicular (FRTL-5) cells. Toxicol In Vitro. 2017;40:297–304.
114. Nieminen P, Lindstrom-Seppa P, Juntunen M, Asikainen J, Mustonen AM, Karonen SL, et al. In vivo effects of bisphenol A on the polecat (mustela putorius). J Toxicol Environ Health A. 2002;65(13):933–45.

Chapter 7
Neurodevelopment and Neurobehavioral Disorders in Relation to Developmental Exposures

Youssef Oulhote and David C. Bellinger

Abstract The environment is now known to be an important determinant of child health, with increasing evidence that some chemicals are particularly toxic to the human brain. More than 140,000 new chemicals have been synthesized since 1950. In this chapter, we review the most studied neurotoxicants for their associations with neurodevelopment and the potential mechanisms of action. We describe the societal effects of such contaminants, and discuss the main challenges facing studies investigating potential neurodevelopmental effects of chemicals. Finally, we provide future directions for the next generation of developmental neuroepidemiology studies.

Keywords Neurodevelopment · Children · Chemicals · Neurotoxicants · Review

Neurodevelopmental disorders are a group of conditions characterized by impairments of social skills or intelligence due to perturbed growth and development of the brain, with onset in the developmental period. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), they include

Y. Oulhote (✉)
Department of Biostatistics and Epidemiology, School of Public Health and Health Sciences, University of Massachusetts Amherst, Amherst, MA, USA

Department of Environmental Health, Harvard T. H. Chan School of Public Health, Boston, MA, USA
e-mail: youlhote@umass.edu

D. C. Bellinger
Department of Environmental Health, Harvard T. H. Chan School of Public Health, Boston, MA, USA

Department of Neurology, Harvard Medical School, Boston, MA, USA

Boston Children's Hospital, Boston, MA, USA
e-mail: David.Bellinger@childrens.harvard.edu

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_7

intellectual disability (intellectual developmental disorder), communication disorders, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD), specific learning disorders, and motor disorders [1]. They are among the most common chronic disorders in children worldwide. For instance, in 2016 9.4% of US children 2–17 years of age had at one time been diagnosed with ADHD [2]. The prevalence of children aged 3–17 years who had ever been diagnosed with a developmental disability increased from 5.8% to 7% between 2014 and 2016 [3], whereas the prevalence of ever receiving an ASD in children aged 3–17 years reached 2.5% in 2016 [4].

Although the conditions included among neurodevelopmental disorders are heterogeneous, these disorders are thought to be developmental, with early onset and involving a multitude of potential risk factors. The environment is now known to be an important determinant of child health, with increasing evidence that some chemicals are particularly toxic to the human brain. More than 140,000 new chemicals and pesticides have been synthesized since 1950, with about 5000 that are produced in high volume, and many have become ubiquitous in the environment [5]. The fetal period is a critical window of development, and in utero exposure to environmental chemicals has a significant influence on fetal growth and development, with consequences for birth outcomes, child development, and cognitive and behavioral functions [6, 7]. For instance, over 200 chemicals used in commerce or industry are known to be neurotoxic to humans [6]. Disruptions caused by exposure to these toxic chemicals may have far-reaching consequences with a considerable impact in regard to long-term complications including developmental delays, effects on socio-emotional adjustment, educational success, and quality of life [8–10]. In this chapter, we describe the accumulated evidence in regard to specific chemicals of interest, the potential mechanisms of action through which exposure to these chemicals affects child neurodevelopment, and the societal impacts of these neurotoxicants. We will finally discuss challenges arising when studying effects, and propose future research needs and directions.

7.1 Main Findings in Regard to Neurodevelopment and Developmental Chemical's Exposure

7.1.1 Trace Elements: Lead, Manganese, Mercury, and Arsenic

There is a compelling evidence that trace metals adversely affect neurodevelopment and increase risk of neurodevelopmental disorders. Lead (Pb) is the most studied environmental toxicant, and its neurotoxic effects have been known and well described for centuries. For instance, Dioscorides, the well-known Greek physician and botanist once said: "Lead makes the mind give away". Thus, Pb is considered a paradigm metal to study. Exposure to high concentrations of Pb has been linked to

effects on the central nervous system, such as deficits in concentration, memory, cognition, and behavior [6, 10]. Epidemiological research has linked Pb exposure during childhood to deficits in cognitive skills and IQ, behavioral problems, and attention hyperactivity disorder (ADHD) [11–14]. Prenatal and early postnatal Pb poisoning and long-term exposure to Pb at low levels has also been associated to an increased risk of aggressive behaviors [15]. Recent studies also showed a link between early-life exposure to Pb and criminal behavior later in life [16]. Overall, Pb exposure unequivocally affects the developing brain, and its effects have been shown for a variety of cognitive and behavioral domains.

Although manganese (Mn) is an essential element, high exposure to Mn has been associated with behavioral problems, decreased cognitive function, and frontal lobe abnormalities [17]. The health implications for fetuses and infants are a concern given the high accumulation of Mn in tissue during development [18]. Indeed, maternal levels of Mn increase markedly during pregnancy, peaking in the third trimester [19]. Both prenatal and postnatal exposure to Mn has been linked to neurodevelopmental effects. In Bangladesh, greater exposure to manganese from drinking water was associated with reduced mathematics achievement scores in school children [20], whereas in Quebec, greater hair concentrations of Mn were associated with hyperactivity [21], reduced memory [22], and attention functions [23]. Other studies from Mexico and Brazil showed reduced intelligence quotient and impaired olfactory function [24, 25].

Arsenic (As) has also received much attention as a neurotoxicant, largely based on studies conducted in Bangladesh. Currently, the permitted concentration of arsenic in water is 10 μg/L (10 ppb); yet, an estimated 100 million people worldwide are exposed to excessive amounts of arsenic via drinking water [26]. As, including inorganic and methylated arsenicals, accumulates in many parts of the brain [27]. Studies of children from Bangladesh showed an inverse association between cognitive function and As levels in water [28, 29]. A recent meta-analysis on the associations between arsenic exposure and IQ concluded that a 50% increase in arsenic levels was associated with a 0.4% decrease in IQ in children [30]. Interestingly, studies on As exposure and neurodevelopment showed differential effects between boys and girls with a higher susceptibility among girls.

Mercury (Hg) is a trace metal of known toxicity. It naturally occurs in several physical and chemical forms, including metallic mercury, inorganic, and organic mercury (e.g., methylmercury, MeHg), with varying toxic effects [31]. Hg can have neurotoxic effects on the human central nervous system, particularly during fetal development. A number of prospective cohort studies assessed the neurodevelopmental effects of chronic low and moderate prenatal MeHg exposure from maternal fish consumption. The most influential studies were conducted in the Faroe Islands and were used by the EPA to establish guidelines for mercury toxicity [6, 32–34].

Although the individual toxicity of these trace elements is now well established, the neurotoxic effects of metals may occur in an interactive way, and several metals may have synergistic effects. However, to date few studies have investigated the independent and joint toxicity of metals within a mixture.

7.1.2 Polychlorinated Biphenyls (PCBs) and Brominated Flame Retardants (PBDEs)

Polychlorinated biphenyls (PCBs) are ubiquitous neurotoxicants used for various industrial applications as coolants and lubricants in electrical equipment because of their general chemical inertness and heat stability [35]. PCBs were manufactured as a mixture of congeners and banned from production and use in the late 1970s. The major exposure route for humans is through food, whereas inhalation and dermal routes are predominant in occupational settings [36]. There is a large body of scientific evidence showing neurodevelopmental effects of early exposure to PCBs. Recent systematic reviews showed that prenatal or early postnatal exposure to PCBs was associated with adverse cognitive and behavior outcomes in most studies, although null associations were also reported [37, 38]. Additionally, a few studies found associations between developmental exposure to PCBs and autism [39, 40].

Polybrominated diphenyl ethers (PBDEs) and their hydroxylated forms have been widely studied for their neurotoxicity. PBDE additives are not fixed in the polymer product through chemical binding, and can thus leak into the environment [41]. Like PCBs, they persist in the environment with relatively long half-lives. Human exposure is mainly due to ingestion of house dust, consumption of fish and other animal products, and breastfeeding for infants [42]. Higher maternal concentrations of PBDEs were associated with impaired cognitive and motor function, increased attention problems, anxious behavior, increased withdrawal, and altered response to frustration [43–46]. Additionally, early-life PBDE exposures were associated with decreased IQ and psychomotor development [47].

7.1.3 Pesticides

There is a strong scientific evidence that exposure to pesticides produces human health effects, including impairment of the central nervous system. Pesticides are widely used in both developed and developing countries, especially with the expansion of free-trade agreements and the focus of many developing countries on agricultural exports. This trend makes extensive pesticides use one of the major environmental health issues in low- and middle-income countries. The most prevalent chemical families of pesticides are organophosphates, organochlorines, and pyrethroids, with organophosphates being the most frequently used. Overall, research points to deleterious effects of pesticides on cognitive and behavioral development in children. Findings from prospective birth cohorts such as the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) point to a multitude of effects of organophosphates, including decreased IQ [48–50], impaired mental development, and pervasive developmental disorder at 24 months of age [51]. A study in New York City found a possible link between prenatal indoor pesticide use and abnormal neonatal reflexes [52]. Other studies from Brittany,

France reported associations between prenatal pesticides exposure in an agricultural area and cranial growth in a prospective birth cohort of 3421 pregnant women [53]. Prenatal exposure to organochlorine pesticides (DDT and its metabolite DDE) was also associated with reduced psychomotor development index scores and significant reduction in the general cognitive index and neurodevelopmental delays [54–56]. However, many studies that investigated the associations between DDE exposure and cognitive and behavioral development reported null findings [57–60]. Unlike DDT, pyrethroids are rapidly metabolized and have short half-lives in humans (hours to days) [61], and very few studies investigated their associations with child neurodevelopment. Oulhote et al. found a positive association between concurrent exposure of some pyrethroid metabolites and child difficulties scores in a cross-sectional study in Canada [62]. Although a French study found no association between prenatal urinary pyrethroid metabolites and 6 year olds' performance on WISC Verbal Comprehension or Working Memory [63], studies from China and Mexico reported that prenatal pyrethroid exposures were associated with lower cognitive scores in 1-year infants [64] and children 2–3 years of age [65]. Further, studies from France and the USA showed adverse effects of pyrethroids exposure on behavioral outcomes in children [66, 67].

7.1.4 Plasticizers

Studies investigating the associations between developmental exposure to plasticizers such as phthalates and bisphenol A (BPA) are still in their infancy but have shown some evidence of associations with both cognitive and behavioral outcomes. Developmental exposure to phthalates was shown to be associated with ADHD behaviors, autistic traits, reduced mental and psychomotor development, and reduced IQ [68–71]. BPA was also reported to be associated with more internalizing and externalizing behaviors, increased risk of ADHD, and executive function [72–75]. Most of these studies point to sexually dimorphic effects of these compounds on child neurodevelopment, with boys being more vulnerable [76]. Other studies showed no association between neither phthalates nor BPA with child neurodevelopment [71, 77].

7.1.5 Perchlorate

Perchlorate is used in a variety of industrial products including missile fuel, fireworks, and fertilizers, and industrial contamination of drinking water occurs in several areas [78]. Exposure to perchlorate is widespread and exposure from contaminated food and drinking water is ubiquitous [79]. Perchlorate can block iodide uptake into the thyroid gland, and result in decreased production and secretion of thyroid hormone, therefore potentially perturbing proper brain development

that is dependent on thyroid function. One study investigated potential neurodevelopmental effects of gestational exposure to perchlorate in the UK and Italy. Findings from this study showed that higher maternal perchlorate levels in hypothyroid/hypothyroxinemic pregnant women were associated with adverse effects on offspring cognitive development [80].

7.2 Potential Mechanisms of Action

Traditionally, mechanisms of neurotoxicity have been identified as pathways leading to neuronal cell death, neuropathology, or neural injury. However, recent research highlights alternative mechanisms that result in more subtle but serious changes in cognition and behavior. These mechanisms include neuroendocrine and immune system pathways, in addition to inflammation and epigenetic mechanisms.

Early-life inflammation induced by exposure to chemicals, especially metals, has been suggested as a potential mechanism for the observed neurodevelopmental effects [81, 82]. For instance, chronic low-level lead exposure may trigger chronic inflammation and lead to adverse changes in inflammatory markers such as CRP [83]. Lead is also known to interfere with the *N*-methyl-D-aspartate receptor, which is essential for hippocampus-mediated learning and memory [84, 85], and to disrupt neurotransmission by inhibiting neuronal voltage-gated calcium channels and intracellular calcium dynamics [86]. Methylmercury also induces impairment in intracellular calcium homeostasis, in addition to alteration of glutamate homeostasis and oxidative stress [87]. Methylmercury perturbs cell proliferation and migration, producing widespread abnormalities including heterotopias, reduced cell densities, incomplete myelination, glial proliferation, and limited gyral differentiation [12]. The effects of Mn on behavior and cognitive abilities in children may be related to effects on the dopaminergic system during development. Mn accumulates in neurons, astrocytes, and oligodendrocytes, inhibits ATP synthesis in mitochondria, and interferes with the dopaminergic system [88, 89]. Arsenic has been shown to impact the synaptic activity of neurons localized to the hippocampus [26]. These effects may be attributable to alterations in synapse-related gene expression. Experimental studies showed that exposure to arsenic throughout gestation increased DNA methylation on two genes involved in neural plasticity in rat cortex and hippocampus [90]. Hypomethylation of these genes in both regions was observed later after 4 months of cumulative exposure to arsenic. Arsenic also interferes with glucocorticoid, cholinergic, and glutamatergic signaling, leading to hippocampal-related deficits [91–93].

Many pesticides, including organophosphates (OPs), carbamates, pyrethroids, and organochlorines (OCs), are designed to attack insects' central nervous system by interfering with chemical neurotransmission or ion channels. They specifically work by targeting enzymes that regulate neurotransmitters, such as acetylcholine, which is released by motor neurons [94]. For instance, both OPs and carbamates have a common target of toxicity, the inhibition of acetylcholinesterase, whose

physiological role is hydrolyzing acetylcholine, a major neurotransmitter in the central and peripheral nervous system [95, 96]. Pyrethroids bind to the sodium channel and slow its activation, as well as the rate of inactivation, leading to a stable hyperexcitable state, and inhibit Gamma aminobutyric $acid_A$-gated chloride channels, leading to the choreoathetosis, salivation, and seizures seen in occupational exposures [96]. Finally, OCs such as DDT interfere with the sodium channels in the axonal membrane, by a mechanism like that of pyrethroids, prolonging the depolarizing (negative) afterpotential of the action potential, thus producing a period of increased neuronal excitability [97].

PCBs exert their neurotoxicity through a variety of mechanisms including thyroid disruption, interference with sex steroids, and aryl hydrocarbon receptor (AhR) activity, especially for dioxin-like PCBs [98–100]. Thyroid and sex steroid hormones are critical for proper brain development, and subtle perturbations of these systems have been shown to be associated with both cognitive and behavioral problems. PBDEs act on the developing brain mainly through a reduction in circulating thyroid hormones; however, direct effects on the developing brain have also been reported. Thyroid hormones are known to play a role in brain development, and a large body of experimental and human studies showed decreased levels of thyroxine (T4) and triiodothyronine (T3), and increased likelihood of hypothyroidism in relation to higher levels of PBDEs [101, 102]. These effects on the thyroid hormone levels are explained by an enhanced metabolism and excretion of T4 as a result of exposure to PBDEs and interactions of PBDEs (or their metabolites) with the thyroid hormone transport systems or with thyroid receptors [103]. In addition to their effect on the thyroid system, PBDEs exert effects by inducing oxidative stress in human neuroblastoma cells and hippocampal neurons, interfering with calcium homeostasis, and causing DNA damage and apoptotic cell death [104–107].

Plasticizers are endocrine-disrupting chemicals that can mimic the effects of endogenous hormones or disrupt the synthesis, metabolism, or uptake of endogenous hormones. For instance, phthalates may interfere with the action or metabolism of androgens and thyroid hormones, with subsequent anti-androgenic effects [108]. Increased concentrations of phthalates were inversely associated with total serum thyroid hormone levels in pregnant women and neonates and thyroid stimulating hormone in neonates [109]. Additionally, phthalate exposure may exert neurotoxic effects by increasing oxidative stress and via epigenetic re-programming of the fetus and placenta [76]. BPA may interfere with thyroid-specific gene expression, and affect androgen/estrogen concentrations by inhibiting key enzymes involved in gonadal hormone synthesis and metabolism [110]. These hormones play a substantial role in brain development [111].

Although perchlorate has not been extensively studied in relation to neurodevelopment, its effect on the thyroid gland through blocking the uptake of iodine is well studied. Low-level exposure to perchlorate has been shown to be positively associated with TSH and negatively associated with free T_4 [112, 113]. Thyroid hormones are essential for proper brain development, and it is therefore hypothesized that the effects of perchlorate on thyroid function may lead to potential neurodevelopmental

consequences [114, 115]. These effects may be stronger in children of women with low iodine status [116].

It is important to mention that these potential mechanisms of action are not mutually exclusive and many chemicals, such as metals, can exhibit endocrine-disrupting properties or increase oxidative stress and inflammation, mediated by epigenetic and neuroendocrine mechanisms.

7.3 Societal Impact of Environmental Chemicals

An argument frequently advanced by those skeptical about the importance of the neurodevelopmental impact of environmental chemicals is that the effect on an individual child is relatively modest, failing to reach the level of clinical significance. This argument fails to consider the issue in the context of population health. Effect estimates from epidemiologic data are population average effects and should be interpreted in the context of a population and not at the individual level. Some individuals will be resistant and some will be more sensitive. It is critical to view the issue of children's exposures to environmental chemicals in the context of population health and not just the health of an individual child. Moreover, the impact of a factor at the population level depends not only on the magnitude of its impact on health, or its effect size, but also on the distribution of the factor or, in the case of a dichotomous factor, its incidence or prevalence. In a set of comparative analyses of pediatric disease and events, such as brain tumors, congenital heart disease, traumatic brain injury, iron deficiency, and lead exposure, Bellinger [97] estimated the total number of IQ points lost among US children younger than 5 years of age associated with each disease or event. The estimate for the loss associated with lead exposure was nearly 23 million IQ points, exceeded only by preterm birth. Among the reasons for this is the absence of a threshold for its inverse relationship with IQ and the fact that virtually every child has a quantifiable blood lead concentration. As a result, and in contrast to most other diseases and events, every child contributes to the total IQ loss in the population that is associated with lead exposure. In fact, the greatest contribution to the total loss is contributed by the very large proportion of children with blood lead concentrations at the lower end of the distribution. A similar calculation using the blood lead distribution of young US children from the late 1970s indicated that, at that time, the total loss of IQ points attributable to lead was approximately 125 million points, suggesting that the measures taken to reduce population lead exposure since that time produced savings of about 100 million IQ points in the current cohort of children. With approximately 25 million children in this age range, the average IQ benefit has been approximately 4 points. Analyzing temporal trends in the IQ scores of US adults over the period in which population blood lead concentrations declined, Kaufman et al. [117] estimated that the mean IQ has increased by 4–5 points.

An even more critical point with regard to the societal impact of environmental chemicals is that a reduction in a child's IQ is only the "tip of the iceberg" in terms of their neurodevelopmental impacts. IQ is easily measured and widely recognized outcome, but it is merely a marker for a range of other neuropsychological and behavioral adversities that have a substantial impact on an individual's future health and well-being. A full account of the burden of disease imposed by environmental chemicals must include these downstream impacts on mental health and economic success that can seriously impair quality of life [12].

This perspective is not captured by the method traditionally used to estimate global burden of diseases and risk factors. Current efforts to estimate the burden of disease associated with environmental chemicals consider only a clinically significant IQ deficit as the sequelae of chemicals such as lead [118], i.e., an IQ score <70, the criterion for identifying mild intellectual disability. As the research reviewed in this chapter illustrates, a reduction in IQ is only the "tip of the iceberg" in terms of the neurodevelopmental impacts of chemicals, which, as described, include deficits in specific neurodevelopmental domains such as executive functions, attention, language, and memory, and mental health disorders such as ADHD and, perhaps, autism spectrum disorders. IQ has the virtue of being easily measured, but an IQ deficit likely is only an indication of the likely presence of a range of other neuropsychological and behavioral adversities that subsequently unfold as a "developmental cascade" and have a substantial impact on an individual's future health and well-being [119].

Another dimension of the societal impact of many environmental chemicals is that the non-random distribution of exposures perpetuates health disparities. In the case of lead, for example, poor and minority children tend to incur greater exposures due to the siting of point sources and the presence of greater lead hazards in the housing available to them. Compounding this problem is the fact that, at any given blood lead concentration, the adverse effects of lead are greater on children already at increased risk of poor outcomes due to the presence of other risk factors. In quantile regression analyses of the associations between blood lead concentration and reading scores, Miranda et al. [120] showed that lead exposure stretched out the left-hand tail of the performance distribution, such that the decrease per μg/dL increase in blood lead concentration was greater among poorer readers than among better readers. One effect would be to aggravate the disparities between the educational outcomes of poor children and their more advantaged peers and, as a study in New Zealand showed, reduce upward social mobility, limiting the socioeconomic success that is achieved in adulthood [121]. The origins of such environmental injustices can be located in the social and economic forces of modern society. One factor contributing to their persistence is the lack of voice affected communities have in influencing those with the power and the means to implement remedies. This was illustrated by the episode of lead contaminated water in Flint, Michigan. It took 18 months after citizens began to complain about the poor quality of their drinking water before government officials acknowledged the problem and began to address it.

7.4 Challenges and Future Directions in Studying Environmental Neurotoxicants

In the following section, we discuss some of the main challenges in investigating the potential neurotoxicity of chemicals. As summarized earlier, many chemicals have been shown to exert neurodevelopmental effects, and the body of literature keeps growing as new compounds are introduced into the market. The multiplicity of environmental neurotoxicants calls for a general framework that considers the cumulative effect of the mixtures, in addition to their potential interactive effects, while including other effect modifiers (i.e., Sociomics). It will also be important that the field adopt novel causal inference approaches that are widely used in clinical, pharmaco-epidemiology, social epidemiology, and econometrics. Finally, the complexity of child development calls for holistic approaches that consider all the facets of development and the multilayers of potential interactions, hence the need to consider broader definitions of the environment beyond just chemicals exposures.

7.4.1 The Issue of Chemical Mixtures and Cumulative Effects

Perhaps one of the most important issues for the field is the development of methods to account for the cumulative effects of chemical mixtures. Although the general population experiences exposure to multiple chemicals from many different sources at various doses, most studies in environmental epidemiology consider each chemical separately when assessing the adverse health effects of environmental exposures. This single pollutant approach suffers from several pitfalls including: (1) the risk of false positives in the case of multiple hypotheses testing; (2) confounding from correlated exposures; and (3) the lack of insights on the cumulative or synergistic effects of multiple exposures. These all weaken the inferences that can be drawn about the relationships between chemicals and neurodevelopmental outcomes. In recent years, several statistical methods have been proposed to address these issues. These methods include environmental-wide association studies (EWAS) [122], penalized regression methods (i.e., least angle selection and shrinkage operator [LASSO]) [123], Ridge regression [124], and elastic net regularization [125], dimension reduction methods, and exposure-response surface methodology such as generalized additive models and Bayesian kernel regression methods (i.e., BKMR) [126]. A recent study by Agier and colleagues used simulation to demonstrate feasibility of some established and emerging methods for handling multiple correlated exposures [127]. While promising, these methods underperformed when the goal was identification of individual exposures with an impact on the phenotype of interest. Many exhibited high false discovery proportions as the number of correlated exposures increased. Moreover, results from a recent NIEHS workshop showed that none of the tested approaches appeared to outperform the others [128], and most of these methods performed poorly in the context of

complex mixtures [127]. More importantly, most of the methods (e.g., elastic net, LASSO) focus on the issues of correlated exposures while ignoring the threats arising from model misspecification (e.g., interactions, non-linearities). The most promising of these methods appears to be the BKMR. This method relaxes a priori parametric assumptions and allows for the investigation of cumulative effects and potential interactions between chemicals. However, one challenge is that such methods provide conditional estimates that are not applicable in simulating public health interventions since they do not provide marginal estimates of the exposures of interest. A new family of methods is emerging that takes advantage of the developments of machine learning, and specifically, ensemble learning techniques (i.e., stacked generalization). Machine-learning methods have shown great potential for quantifying the role of environmental exposures in regard to their effects on human health [129]. Typically, machine-learning approaches consist of algorithms that find variables (exposures) that are predictive of an outcome (phenotype) in two steps. In the first step, an algorithm "learns" the variables that are associated with the outcome. The algorithm is then tested in an independent dataset to estimate the predictive capability or generalizability of the algorithm [130]. Ensemble techniques reduce the variance and avoid overfitting by combining predictions from numerous "similar" algorithms. Although these methods are in their infancy, several of them have shown promising results in the context of chemical mixtures [131, 132], while others have been used in other contexts and could be leveraged to infer causal estimates of complex mixtures [133]. These methods allow for incorporation of multiple correlated exposures, estimation of both individual and cumulative effects at the population level, and screening of potential interactions. Moreover, these ensemble learning methods allow for more flexibility since they relax a priori assumptions regarding the functional forms and presence of interactions, which means that one can include all the exposures without specifying interactions or functional forms (e.g., non-linearities) and the approach will identify the models that yield the best predictions.

7.4.2 Incorporating Causal Inference Methods When Studying Neurotoxicants

For obvious ethical reasons, it is often impossible to conduct randomized controlled trials in the field of environmental epidemiology. Therefore, most studies investigating potential neurodevelopmental effects of chemicals rely on observational studies. Additionally, other issues arise when studying environmental chemicals that make causal inferences difficult. First, exposure to the chemical of interest is typically ubiquitous, and all individuals in the study are exposed at a detectable level, leading to continuous exposures, making emulation of RCTs nearly impossible. Second, environmental epidemiology relies on measurements of biomarkers to investigate the exposure-outcome relationship; such reliance on biomarkers makes it difficult to

insure "consistency" and "positivity," two major assumptions necessary for inferring causal effects. "The positivity assumption states that there is a nonzero probability of receiving every level of exposure for every combination of values of exposure and confounders that occur among individuals in the population" [134]. In other words, within each level of the exposure, one will need individuals with all levels of all confounders. This is obviously extremely difficult in studying continuous exposures unless a cohort includes millions of participants or exposures are categorized. The consistency assumption is the ability to hypothetically assign a certain level of exposure to a person exposed to a different level. This opens to the issue of defining what intervention could hypothetically lower the measured biomarker level to a specific desired level in an individual, or what other methodologists call "well-defined interventions" [135]. This calls for the identification of what kind of interventions can directly change the concentrations of a neurotoxicant in the tissue of interest (or circulating blood levels that are also just proxies of the level of neurotoxicant in the target tissue) [136]. This is obviously impossible outside of a few cases (e.g., chelation treatment for lead). Many physiological parameters will influence the measures of circulating levels of a neurotoxicant, and therefore impact the relationship between the external intervention (on which we can intervene) and the biomarker concentrations. This will likely violate the consistency assumption thereby impeding valid causal estimates. However, it is worth mentioning that this issue of consistency can be challenged in terms of scientific discovery. Not all studies aim at emulating an intervention when studying health effects of chemicals, therefore, the knowledge acquired from establishing causal links between a biomarker of exposure and neurodevelopment is still valuable and will likely lead to measures that can reduce external exposures at the population level. For more details on this debate, reader can refer to papers by Judea Pearl, Miguel Hernan, and others [135, 137, 138].

There is also a new body of methods that have been proposed recently to tackle some of the issues arising when inferring causality from observational data. One of the promising methods that can be used is Mendelian randomization analysis [139], which uses genetic variants as instrumental variables for exposures of interest. It is a useful method for assessing causal relationships because the allocation of alleles is random; therefore, genotypes are not expected to be associated with measured or unmeasured confounders. The use of single nucleotide polymorphisms (SNPs) that are associated with exposure levels but not with the outcome of interest (except through the exposure) represents an appropriate approach to examine the causal relationship between pollutant exposures and children's developmental outcomes. The metabolism and excretion of many xenobiotic substances has been linked to many SNPs (e.g., pesticides and paraoxonase or cytochrome P450). Using these SNPs to construct allelic scores robustly related to the exposures as instrumental variables may help to identify causal effects of environmental exposures on neurodevelopmental test scores. One limitation of such approaches is the lack of instrumental variables (SNPs) that are specific to a class of chemicals, but research on this field should yield valid instruments in the coming years. One study used two SNPs in the 10q24.32 region (near AS3MT) that show independent associations with arsenic metabolism efficiency to investigate the causal relationship between arsenic

metabolism efficiency and skin lesion risk [140]. Another issue that arises when using such methods is that they rely on very large sample sizes, and this will depend on the strength of the association between the SNP (or the allelic score combining many SNPs) and the biomarker of exposure. The stronger the association, the smaller the sample size that is needed.

Additional methods that can be leveraged are econometric methods that rely on quasi-experimental designs. Some of these methods leverage natural experiments to emulate randomized controlled trials. We recently leveraged the fact that undergrad students at Harvard University are randomly assigned to dormitories to infer causal estimates of high temperatures on cognitive functions among students during a heat wave [141]. A recent study from the National Bureau of Economic Research showed causal effects of air pollution on dementia by capitalizing on quasi-random variation in pollution exposure due to the EPA's 2005 designation of nonattainment counties for $PM_{2.5}$ [142]. Closer to our field, some mesalamine medications used to treat inflammatory bowel disease have dibutyl-phthalate (DBP) in their coating, whereas other mesalamine formulations do not. This difference and the fact that the type of medication depends mainly on the prescribing physician and not on other potential confounders have been leveraged to study the potential effect of DBP on semen quality [143]. Creativity in exploiting such random events will be a very useful approach in solving some of the most challenging issues in environmental neuroepidemiology studies.

7.4.3 Measurement Error in Exposures

Perhaps one of the most critical issues in the field of environmental epidemiology is the error in measuring both exposures and outcomes. Exposure and outcome misclassification can seriously affect estimates from studies investigating neurodevelopmental effects of chemicals. Non-differential exposure misclassification is a common problem, especially for endocrine-disrupting chemicals with short half-lives that tend to vary widely within individual. Under such conditions, an individual's true exposure will be difficult to establish because of the low precision of the measurement. When error is not related to the outcome of interest, this will often, but not always, bias the association towards the null. Several studies have used multiple samples to characterize exposure to short half-life compounds, yielding a better characterization. However, this will require a large number of samples, increasing costs in terms of follow-up and analyses [144]. Other researchers proposed using calibration and Bayesian methods to account for non-differential misclassification errors, but these are very rarely used [145]. Construction of latent functions based on measurements of a chemical in multiple matrices represents a hypothetical true measure of exposure and could tackle this issue by modeling the shared variance from these multiple measures instead of a single concentration. This approach was used in studies to examine neurodevelopmental effects of mercury and PFASs [23, 33, 146]. Other researchers propose sensitivity analyses to explore the impact of such biases, allowing for inference of interval estimates.

7.4.4 Holistic Approaches to Study Child Development

Child neurodevelopment is a dynamic process with a complex structure of determinants and feedback loops. Present studies of developmental neurotoxicants are still in their infancy in terms of addressing such complexity and future studies may benefit from incorporating it both in the study design and analyses stages. Currently, there is a strong need for a comprehensive approach that puts epidemiological studies of neurotoxicants within a developmental framework, particularly across the lifespan.

Context information matters and is a key to understanding effects of neurotoxicants on child development. For instance, many non-environmental cofactors play a key role beyond their main effects and can be effect modifiers of the associations between neurotoxicants and neurodevelopment. Many confounders can potentially play an effect modification role. Additionally, every factor that is impacting child neurodevelopment and not associated with the exposure may modify neurotoxicant's effect. An important example is the stimulating home environment. While it is very important to take this variable into account, it is not acting as a confounder in most of the studies, but mainly as a strong predictor of the neurodevelopmental outcome. It is therefore very useful to test for its modification effect. Another important example is some nutritional factors that co-occur with environmental exposures. Recently, a few studies showed that folic acid supplementation may blunt the deleterious effects of pesticides and air pollutants on autism [147, 148]. Selenium has also been shown to play an important modifying role in the association between mercury and neurodevelopment [149]. Both these examples of effect modifiers including stimulation and nutritional factors show up when investigating effect modification by socioeconomic status.

These contextual factors may be of an utmost interest in areas of the globe in which many environmental, nutritional, and social factors co-act to yield a high burden of developmental disabilities. For instance, around a third of children in low- and middle-income countries are not developmentally on track [150]. The interplay between nutritional deficiencies, lack of child stimulation due to education policies and early childcare, and a high burden of environmental exposures may explain a significant part of the observed delay in children's neurodevelopment in these countries. These issues cannot be tackled separately, hence the need for holistic approaches to study neurotoxicants, in addition to an urgent need to develop specific and tailored frameworks that can incorporate these complexities. Transposition of western models to the LMIC realities may likely fail if local dynamics and holistic approaches to environmental health are not considered.

References

1. Harris JC. New classification for neurodevelopmental disorders in DSM-5. Curr Opin Psychiatry. 2014;27(2):95–7.
2. Danielson ML, Bitsko RH, Ghandour RM, Holbrook JR, Kogan MD, Blumberg SJ. Prevalence of parent-reported ADHD diagnosis and associated treatment among U.S. children and adolescents, 2016. J Clin Child Adolesc Psychol. 2018;47(2):199–212.

3. Zablotsky B, Black LI, Blumberg SJ. Estimated prevalence of children with diagnosed developmental disabilities in the United States, 2014-2016. NCHS Data Brief. 2017;291:1–8.
4. Kogan MD, Vladutiu CJ, Schieve LA, Ghandour RM, Blumberg SJ, Zablotsky B, et al. The prevalence of parent-reported autism spectrum disorder among US children. Pediatrics. 2018;142(6):e20174161.
5. Landrigan PJ, Fuller R, Acosta NJR, Adeyi O, Arnold R, Basu NN, et al. The Lancet Commission on pollution and health. Lancet. 2017;391(10119):462–512.
6. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol. 2014;13(3):330–8.
7. Mattison DR. Environmental exposures and development. Curr Opin Pediatr. 2010;22(2):208–18.
8. Zwicker JG, Harris SR. Quality of life of formerly preterm and very low birth weight infants from preschool age to adulthood: a systematic review. Pediatrics. 2008;121(2):e366–76.
9. Moore T, Hennessy EM, Myles J, Johnson SJ, Draper ES, Costeloe KL, et al. Neurological and developmental outcome in extremely preterm children born in England in 1995 and 2006: the epicure studies. BMJ. 2012;68:345.
10. Rauh VA, Margolis AE. Research Review: environmental exposures, neurodevelopment and child mental health – new paradigms for the study of brain and behavioral effects. J Child Psychol Psychiatry. 2016;57(7):775–93.
11. Bellinger D, Leviton A, Allred E, Rabinowitz M. Pre- and postnatal lead exposure and behavior problems in school-aged children. Environ Res. 1994;66(1):12–30.
12. Bellinger DC. Environmental chemical exposures and neurodevelopmental impairments in children. Pediatr Med. 2018;2018:1.
13. Chen A, Cai B, Dietrich KN, Radcliffe J, Rogan WJ. Lead exposure, IQ, and behavior in urban 5- to 7-year-olds: does lead affect behavior only by lowering IQ? Pediatrics. 2007;119(3):e650–e8.
14. Eubig PA, Aguiar A, Schantz SL. Lead and PCBs as risk factors for attention deficit/hyperactivity disorder. Environ Health Perspect. 2010;118(12):1654–67.
15. Nkomo P, Naicker N, Mathee A, Galpin J, Richter LM, Norris SA. The association between environmental lead exposure with aggressive behavior, and dimensionality of direct and indirect aggression during mid-adolescence: Birth to Twenty Plus Cohort. Sci Total Environ. 2018;612:472–9.
16. Wright JP, Dietrich KN, Ris MD, Hornung RW, Wessel SD, Lanphear BP, et al. Association of prenatal and childhood blood lead concentrations with criminal arrests in early adulthood. PLoS Med. 2008;5(5):e101.
17. Guilarte TR. Manganese neurotoxicity: new perspectives from behavioral, neuroimaging, and neuropathological studies in humans and non-human primates. Front Aging Neurosci. 2013;5:23.
18. Fechter LD. Distribution of manganese in development. Neurotoxicology. 1999;20(2-3):197–201.
19. Takser L, Lafond J, Bouchard M, St-Amour G, Mergler D. Manganese levels during pregnancy and at birth: relation to environmental factors and smoking in a Southwest Quebec population. Environ Res. 2004;95(2):119–25.
20. Khan K, Wasserman GA, Liu X, Ahmed E, Parvez F, Slavkovich V, et al. Manganese exposure from drinking water and children's academic achievement. Neurotoxicology. 2012;33(1):91–7.
21. Bouchard M, Laforest F, Vandelac L, Bellinger D, Mergler D. Hair manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. Environ Health Perspect. 2007;115(1):122–7.
22. Carvalho CF, Oulhote Y, Martorelli M, Carvalho CO, Menezes-Filho JA, Argollo N, et al. Environmental manganese exposure and associations with memory, executive functions, and hyperactivity in Brazilian children. Neurotoxicology. 2018;69:253–9.
23. Oulhote Y, Mergler D, Barbeau B, Bellinger DC, Bouffard T, Brodeur ME, et al. Neurobehavioral function in school-age children exposed to manganese in drinking water. Environ Health Perspect. 2014;122(12):1343–50.

24. Ortiz-Romo N, Drucker-Colín R, Guarneros M, Alcaraz-Zubeldia M, Hudson R. Nonoccupational environmental exposure to manganese is linked to deficits in peripheral and central olfactory function. Chem Senses. 2013;38(9):783–91.
25. Carvalho CF, Menezes-Filho JA, de Matos VP, Bessa JR, Coelho-Santos J, Viana GF, et al. Elevated airborne manganese and low executive function in school-aged children in Brazil. Neurotoxicology. 2014;45:301–8.
26. Tyler CR, Allan AM. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr Environ Health Rep. 2014;1(2):132–47.
27. Sanchez-Pena LC, Petrosyan P, Morales M, Gonzalez NB, Gutierrez-Ospina G, Del Razo LM, et al. Arsenic species, AS3MT amount, and AS3MT gene expression in different brain regions of mouse exposed to arsenite. Environ Res. 2010;110(5):428–34.
28. Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, et al. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect. 2004;112(13):1329–33.
29. Wasserman GA, Liu X, Parvez F, Factor-Litvak P, Kline J, Siddique AB, et al. Child intelligence and reductions in water arsenic and manganese: a two-year follow-up study in Bangladesh. Environ Health Perspect. 2016;124(7):1114–20.
30. Rodriguez-Barranco M, Lacasana M, Aguilar-Garduno C, Alguacil J, Gil F, Gonzalez-Alzaga B, et al. Association of arsenic, cadmium and manganese exposure with neurodevelopment and behavioural disorders in children: a systematic review and meta-analysis. Sci Total Environ. 2013;454-455:562–77.
31. Moneim AEA. Mercury-induced neurotoxicity and neuroprotective effects of berberine. Neural Regen Res. 2015;10(6):881–2.
32. Debes F, Budtz-Jorgensen E, Weihe P, White RF, Grandjean P. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. Neurotoxicol Teratol. 2006;28(5):536–47.
33. Oulhote Y, Debes F, Vestergaard S, Weihe P, Grandjean P. Aerobic fitness and neurocognitive function scores in young faroese adults and potential modification by prenatal methylmercury exposure. Environ Health Perspect. 2017;125(4):677–83.
34. Grandjean P, White RF. Effects of methylmercury exposure on neurodevelopment. JAMA. 1999;281(10):896.
35. Hopf NB, Ruder AM, Succop P. Background levels of polychlorinated biphenyls in the U.S. population. Sci Total Environ. 2009;407(24):6109–19.
36. Faroon O, Ruiz P. Polychlorinated biphenyls: new evidence from the last decade. Toxicol Ind Health. 2016;32(11):1825–47.
37. Berghuis SA, Bos AF, Sauer PJJ, Roze E. Developmental neurotoxicity of persistent organic pollutants: an update on childhood outcome. Arch Toxicol. 2015;89(5):687–709.
38. Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. Environmental pollutants and child health—a review of recent concerns. Int J Hyg Environ Health. 2016;219(4):331–42.
39. Lyall K, Schmidt RJ, Hertz-Picciotto I. Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol. 2014;43(2):443–64.
40. Rossignol DA, Genuis SJ, Frye RE. Environmental toxicants and autism spectrum disorders: a systematic review. Transl Psychiatry. 2014;4:e360.
41. Costa LG, Giordano G. Developmental neurotoxicity of polybrominated diphenyl ether (PBDE) flame retardants. Neurotoxicology. 2007;28(6):1047–67.
42. Hendriks H, Westerink R. Neurotoxicity and risk assessment of brominated and alternative flame retardants. Neurotoxicol Teratol. 2015;52(Pt B):248–69.
43. Herbstman JB, Sjödin A, Kurzon M, Lederman SA, Jones RS, Rauh V, et al. Prenatal exposure to PBDEs and neurodevelopment. Environ Health Perspect. 2010;118(5):712–9.
44. Hudson-Hanley B, Irvin V, Flay B, MacDonald M, Kile ML. Prenatal PBDE exposure and neurodevelopment in children 7 years old or younger: a systematic review and meta-analysis. Curr Epidemiol Rep. 2018;5(1):46–59.
45. Sagiv SK, Kogut K, Gaspar FW, Gunier RB, Harley KG, Parra K, et al. Prenatal and childhood polybrominated diphenyl ether (PBDE) exposure and attention and executive function at 9–12 years of age. Neurotoxicol Teratol. 2015;52:151–61.

46. Oulhote Y, Tremblay E, Arbuckle TE, Fraser WD, Lemelin JP, Seguin JR, et al. Prenatal exposure to polybrominated diphenyl ethers and predisposition to frustration at 7 months: results from the MIREC study. Environ Int. 2018;119:79–88.
47. Herbstman JB, Mall JK. Developmental exposure to polybrominated diphenyl ethers and neurodevelopment. Curr Environ Health Rep. 2014;1(2):101–12.
48. Bouchard MF, Chevrier J, Harley KG, Kogut K, Vedar M, Calderon N, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect. 2011;119(8):1189–95.
49. Engel Stephanie M, Wetmur J, Chen J, Zhu C, Barr Dana B, Canfield Richard L, et al. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ Health Perspect. 2011;119(8):1182–8.
50. Rauh V, Arunajadai S, Horton M, Perera F, Hoepner L, Barr Dana B, et al. Seven-year neurodevelopmental scores and prenatal exposure to chlorpyrifos, a common agricultural pesticide. Environ Health Perspect. 2011;119(8):1196–201.
51. Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect. 2007;115(5):792–8.
52. Engel SM, Berkowitz GS, Barr DB, Teitelbaum SL, Siskind J, Meisel SJ, et al. Prenatal organophosphate metabolite and organochlorine levels and performance on the Brazelton Neonatal Behavioral Assessment Scale in a multiethnic pregnancy cohort. Am J Epidemiol. 2007;165(12):1397–404.
53. Petit C, Chevrier C, Durand G, Monfort C, Rouget F, Garlantezec R, et al. Impact on fetal growth of prenatal exposure to pesticides due to agricultural activities: a prospective cohort study in Brittany, France. Environ Health. 2010;9:71.
54. Torres-Sanchez L, Rothenberg SJ, Schnaas L, Cebrian ME, Osorio E, Del Carmen HM, et al. In utero p,p′-DDE exposure and infant neurodevelopment: a perinatal cohort in Mexico. Environ Health Perspect. 2007;115(3):435–9.
55. Torres-Sánchez L, Schnaas L, Rothenberg SJ, Cebrián ME, Osorio-Valencia E, Hernández MDC, et al. Prenatal p,p′-DDE exposure and neurodevelopment among children 3.5-5 years of age. Environ Health Perspect. 2013;121(2):263–8.
56. Eskenazi B, Marks AR, Bradman A, Fenster L, Johnson C, Barr DB, et al. In utero exposure to dichlorodiphenyltrichloroethane (DDT) and dichlorodiphenyldichloroethylene (DDE) and neurodevelopment among young Mexican American children. Pediatrics. 2006;118(1):233–41.
57. Eskenazi B, An S, Rauch SA, Coker ES, Maphula A, Obida M, et al. Prenatal exposure to DDT and pyrethroids for malaria control and child neurodevelopment: The VHEMBE Cohort, South Africa. Environ Health Perspect. 2018;126(4):047004.
58. Gaspar FW, Harley KG, Kogut K, Chevrier J, Mora AM, Sjodin A, et al. Prenatal DDT and DDE exposure and child IQ in the CHAMACOS cohort. Environ Int. 2015;85:206–12.
59. Pan IJ, Daniels JL, Goldman BD, Herring AH, Siega-Riz AM, Rogan WJ. Lactational exposure to polychlorinated biphenyls, dichlorodiphenyltrichloroethane, and dichlorodiphenyldichloroethylene and infant neurodevelopment: an analysis of the pregnancy, infection, and nutrition babies study. Environ Health Perspect. 2009;117(3):488–94.
60. Jusko TA, Klebanoff MA, Brock JW, Longnecker MP. In-utero exposure to dichlorodiphenyltrichloroethane and cognitive development among infants and school-aged children. Epidemiology. 2012;23(5):689–98.
61. Barlow SM, Sullivan FM, Lines J. Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. Food Chem Toxicol. 2001;39(5):407–22.
62. Oulhote Y, Bouchard MF. Urinary metabolites of organophosphate and pyrethroid pesticides and behavioral problems in Canadian children. Environ Health Perspect. 2013;121(11-12):1378–84.
63. Viel JF, Warembourg C, Le Maner-Idrissi G, Lacroix A, Limon G, Rouget F, et al. Pyrethroid insecticide exposure and cognitive developmental disabilities in children: The PELAGIE Mother-Child Cohort. Environ Int. 2015;82:69–75.

64. Xue Z, Li X, Su Q, Xu L, Zhang P, Kong Z, et al. Effect of synthetic pyrethroid pesticide exposure during pregnancy on the growth and development of infants. Asia Pac J Public Health. 2013;25(4 Suppl):72s–9s.
65. Watkins DJ, Fortenberry GZ, Sánchez BN, Barr DB, Panuwet P, Schnaas L, et al. Urinary 3-phenoxybenzoic acid (3-PBA) levels among pregnant women in Mexico City: distribution and relationships with child neurodevelopment. Environ Res. 2016;147:307–13.
66. Viel JF, Rouget F, Warembourg C, Monfort C, Limon G, Cordier S, et al. Behavioural disorders in 6-year-old children and pyrethroid insecticide exposure: The PELAGIE Mother-Child Cohort. Occup Environ Med. 2017;74(4):275–81.
67. Furlong MA, Barr DB, Wolff MS, Engel SM. Prenatal exposure to pyrethroid pesticides and childhood behavior and executive functioning. Neurotoxicology. 2017;62:231–8.
68. Factor-Litvak P, Insel B, Calafat AM, Liu X, Perera F, Rauh VA, et al. Persistent associations between maternal prenatal exposure to phthalates on child IQ at age 7 years. PLoS One. 2014;9(12):e114003.
69. Arbuckle TE, Davis K, Boylan K, Fisher M, Fu J. Bisphenol A, phthalates and lead and learning and behavioral problems in Canadian children 6–11 years of age: CHMS 2007–2009. Neurotoxicology. 2016;54:89–98.
70. Kim BN, Cho SC, Kim Y, Shin MS, Yoo HJ, Kim JW, et al. Phthalates exposure and attention-deficit/hyperactivity disorder in school-age children. Biol Psychiatry. 2009;66(10):958–63.
71. Miodovnik A, Engel SM, Zhu C, Ye X, Soorya LV, Silva MJ, et al. Endocrine disruptors and childhood social impairment. Neurotoxicology. 2011;32(2):261–7.
72. Harley KG, Gunier RB, Kogut K, Johnson C, Bradman A, Calafat AM, et al. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ Res. 2013;126:43–50.
73. Roen EL, Wang Y, Calafat AM, Wang S, Margolis A, Herbstman J, et al. Bisphenol A exposure and behavioral problems among inner city children at 7–9 years of age. Environ Res. 2015;142:739–45.
74. Evans SF, Kobrosly RW, Barrett ES, Thurston SW, Calafat AM, Weiss B, et al. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. Neurotoxicology. 2014;45:91–9.
75. Casas M, Forns J, Martinez D, Avella-Garcia C, Valvi D, Ballesteros-Gomez A, et al. Exposure to bisphenol A during pregnancy and child neuropsychological development in the INMA-Sabadell cohort. Environ Res. 2015;142:671–9.
76. Braun JM. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nat Rev Endocrinol. 2016;13:161.
77. Braun JM, Kalkbrenner AE, Just AC, Yolton K, Calafat AM, Sjodin A, et al. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: The HOME Study. Environ Health Perspect. 2014;122(5):513–20.
78. Steinmaus CM. Perchlorate in water supplies: sources, exposures, and health effects. Curr Environ Health Rep. 2016;3(2):136–43.
79. Blount BC, Valentin-Blasini L. Biomonitoring as a method for assessing exposure to perchlorate. Thyroid. 2007;17(9):837–41.
80. Taylor PN, Okosieme OE, Murphy R, Hales C, Chiusano E, Maina A, et al. Maternal perchlorate levels in women with borderline thyroid function during pregnancy and the cognitive development of their offspring: data from the controlled antenatal thyroid study. J Clin Endocrinol Metabol. 2014;99(11):4291–8.
81. Fry RC, Navasumrit P, Valiathan C, Svensson JP, Hogan BJ, Luo M, et al. Activation of inflammation/NF-kappaB signaling in infants born to arsenic-exposed mothers. PLoS Genet. 2007;3(11):e207.
82. Dietert RR. Misregulated inflammation as an outcome of early-life exposure to endocrine-disrupting chemicals. Rev Environ Health. 2012;27(2-3):117–31.
83. Sirivarasai J, Wananukul W, Kaojarern S, Chanprasertyothin S, Thongmung N, Ratanachaiwong W, et al. Association between inflammatory marker, environmental lead exposure, and glutathione S-transferase gene. Biomed Res Int. 2013;2013:474963.

84. Morris RG, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature. 1986;319(6056):774–6.
85. Toscano CD, Guilarte TR. Lead neurotoxicity: from exposure to molecular effects. Brain Res. 2005;49(3):529–54.
86. Neal AP, Guilarte TR. Mechanisms of lead and manganese neurotoxicity. Toxicol Res. 2013;2(2):99–114.
87. Farina M, Rocha JBT, Aschner M. Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies. Life Sci. 2011;89(15-16):555–63.
88. Gavin CE, Gunter KK, Gunter TE. Mn2+ sequestration by mitochondria and inhibition of oxidative phosphorylation. Toxicol Appl Pharmacol. 1992;115(1):1–5.
89. Burton NC, Guilarte TR. Manganese neurotoxicity: lessons learned from longitudinal studies in nonhuman primates. Environ Health Perspect. 2009;117(3):325–32.
90. Martinez L, Jimenez V, Garcia-Sepulveda C, Ceballos F, Delgado JM, Nino-Moreno P, et al. Impact of early developmental arsenic exposure on promotor CpG-island methylation of genes involved in neuronal plasticity. Neurochem Int. 2011;58(5):574–81.
91. Martinez-Finley EJ, Ali AM, Allan AM. Learning deficits in C57BL/6J mice following perinatal arsenic exposure: consequence of lower corticosterone receptor levels? Pharmacol Biochem Behav. 2009;94(2):271–7.
92. Rodriguez VM, Carrizales L, Mendoza MS, Fajardo OR, Giordano M. Effects of sodium arsenite exposure on development and behavior in the rat. Neurotoxicol Teratol. 2002;24(6):743–50.
93. Kruger K, Binding N, Straub H, Musshoff U. Effects of arsenite on long-term potentiation in hippocampal slices from young and adult rats. Toxicol Lett. 2006;165(2):167–73.
94. Gilden RC, Huffling K, Sattler B. Pesticides and health risks. JOGNN. 2010;39(1):103–10.
95. Costa LG. Interactions of neurotoxicants with neurotransmitter systems. Toxicology. 1988;49(2):359–66.
96. Costa LG, Giordano G, Guizzetti M, Vitalone A. Neurotoxicity of pesticides: a brief review. Front Biosci. 2008;13:1240–9.
97. Vijverberg HP, van der Zalm JM, van der Bercken J. Similar mode of action of pyrethroids and DDT on sodium channel gating in myelinated nerves. Nature. 1982;295(5850):601–3.
98. Tilson HA, Kodavanti PR. The neurotoxicity of polychlorinated biphenyls. Neurotoxicology. 1998;19(4-5):517–25.
99. Schantz SL. Developmental neurotoxicity of PCBs in humans: what do we know and where do we go from here? Neurotoxicol Teratol. 1996;18(3):217–27.
100. Ribas-Fitó N, Sala M, Kogevinas M, Sunyer J. Polychlorinated biphenyls (PCBs) and neurological development in children: a systematic review. J Epidemiol Community Health. 2001;55(8):537–46.
101. Oulhote Y, Chevrier J, Bouchard MF. Exposure to polybrominated diphenyl ethers (PBDEs) and hypothyroidism in Canadian women. J Clin Endocrinol Metab. 2016;101(2):590–8.
102. Chevrier J, Harley KG, Bradman A, Gharbi M, Sjodin A, Eskenazi B. Polybrominated diphenyl ether (PBDE) flame retardants and thyroid hormone during pregnancy. Environ Health Perspect. 2010;118(10):1444–9.
103. Costa LG, de Laat R, Tagliaferri S, Pellacani C. A mechanistic view of polybrominated diphenyl ether (PBDE) developmental neurotoxicity. Toxicol Lett. 2014;230(2):282–94.
104. Blanco J, Mulero M, Lopez M, Domingo JL, Sanchez DJ. BDE-99 deregulates BDNF, Bcl-2 and the mRNA expression of thyroid receptor isoforms in rat cerebellar granular neurons. Toxicology. 2011;290(2-3):305–11.
105. Tagliaferri S, Caglieri A, Goldoni M, Pinelli S, Alinovi R, Poli D, et al. Low concentrations of the brominated flame retardants BDE-47 and BDE-99 induce synergistic oxidative stress-mediated neurotoxicity in human neuroblastoma cells. Toxicol In Vitro. 2010;24(1):116–22.
106. Madia F, Giordano G, Fattori V, Vitalone A, Branchi I, Capone F, et al. Differential in vitro neurotoxicity of the flame retardant PBDE-99 and of the PCB Aroclor 1254 in human astrocytoma cells. Toxicol Lett. 2004;154(1-2):11–21.

107. Mariussen E, Fonnum F. Neurochemical targets and behavioral effects of organohalogen compounds: an update. Crit Rev Toxicol. 2006;36(3):253–89.
108. Talsness CE, Andrade AJM, Kuriyama SN, Taylor JA, vom Saal FS. Components of plastic: experimental studies in animals and relevance for human health. Philos Trans R Soc London Ser B. 2009;364(1526):2079–96.
109. Romano ME, Eliot MN, Zoeller RT, Hoofnagle AN, Calafat AM, Karagas MR, et al. Maternal urinary phthalate metabolites during pregnancy and thyroid hormone concentrations in maternal and cord sera: The HOME Study. Int J Hyg Environ Health. 2018;221(4):623–31.
110. Zhang X, Chang H, Wiseman S, He Y, Higley E, Jones P, et al. Bisphenol A disrupts steroidogenesis in human H295R cells. Toxicol Sci. 2011;121(2):320–7.
111. Gentilcore D, Porreca I, Rizzo F, Ganbaatar E, Carchia E, Mallardo M, et al. Bisphenol A interferes with thyroid specific gene expression. Toxicology. 2013;304:21–31.
112. Blount BC, Pirkle JL, Osterloh JD, Valentin-Blasini L, Caldwell KL. Urinary perchlorate and thyroid hormone levels in adolescent and adult men and women living in the United States. Environ Health Perspect. 2006;114(12):1865–71.
113. Charatcharoenwitthaya N, Ongphiphadhanakul B, Pearce EN, Somprasit C, Chanthasenanont A, He X, et al. The association between perchlorate and thiocyanate exposure and thyroid function in first-trimester pregnant Thai women. J Clin Endocrinol Metabol. 2014;99(7): 2365–71.
114. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 1999;341(8):549–55.
115. Li Y, Shan Z, Teng W, Yu X, Li Y, Fan C, et al. Abnormalities of maternal thyroid function during pregnancy affect neuropsychological development of their children at 25–30 months. Clin Endocrinol. 2010;72(6):825–9.
116. Trumbo PR. Perchlorate consumption, iodine status, and thyroid function. Nutr Rev. 2010;68(1):62–6.
117. Kaufman AS, Zhou X, Reynolds MR, Kaufman NL, Green GP, Weiss LG. The possible societal impact of the decrease in U.S. blood lead levels on adult IQ. Environ Res. 2014;132:413–20.
118. Collaborators GDaIIaP. Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet. 2017;390(10100):1211–59.
119. Bellinger DC, Matthews-Bellinger JA, Kordas K. A developmental perspective on early-life exposure to neurotoxicants. Environ Int. 2016;94:103–12.
120. Miranda ML, Kim D, Reiter J, Overstreet Galeano MA, Maxson P. Environmental contributors to the achievement gap. Neurotoxicology. 2009;30(6):1019–24.
121. Reuben A, Caspi A, Belsky DW, Broadbent J, Harrington H, Sugden K, et al. Association of childhood blood lead levels with cognitive function and socioeconomic status at age 38 years and with IQ change and socioeconomic mobility between childhood and adulthood. JAMA. 2017;317(12):1244–51.
122. Patel CJ, Bhattacharya J, Butte AJ. An environment-wide association study (EWAS) on type 2 diabetes mellitus. PLoS One. 2010;5(5):e10746.
123. Tibshirani R. Regression shrinkage and selection via the lasso. J R Stat Soc Ser B. 1996;58(1):267–88.
124. Hoerl AE, Kennard RW. Ridge regression: biased estimation for nonorthogonal problems. Technometrics. 1970;12(1):55–67.
125. Zou H, Hastie T. Regularization and variable selection via the elastic net. J R Stat Soc. 2005;67(2):301–20.
126. Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, et al. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics. 2014;16(3):493–508.

127. Agier L, Portengen L, Chadeau-Hyam M, Basagana X, Giorgis-Allemand L, Siroux V, et al. A systematic comparison of linear regression-based statistical methods to assess exposome-health associations. Environ Health Perspect. 2016;124(12):1848–56.
128. Taylor KW, Joubert BR, Braun JM, Dilworth C, Gennings C, Hauser R, et al. Statistical approaches for assessing health effects of environmental chemical mixtures in epidemiology: lessons from an innovative workshop. Environ Health Perspect. 2016;124(12):A227–a9.
129. Sun Z, Tao Y, Li S, Ferguson KK, Meeker JD, Park SK, et al. Statistical strategies for constructing health risk models with multiple pollutants and their interactions: possible choices and comparisons. Environ Health. 2013;12(1):85.
130. Patel CJ. Analytic complexity and challenges in identifying mixtures of exposures associated with phenotypes in the exposome era. Curr Epidemiol Rep. 2017;4:22–30.
131. Oulhote Y, Bind M-A, Coull B, Patel CJ, Grandjean P. Combining ensemble learning techniques and G-computation to investigate chemical mixtures in environmental epidemiology studies. bioRxiv:147413. 2017.
132. Park SK, Zhao Z, Mukherjee B. Construction of environmental risk score beyond standard linear models using machine learning methods: application to metal mixtures, oxidative stress and cardiovascular disease in NHANES. Environ Health. 2017;16(1):102.
133. Kennedy EH, Ma Z, McHugh MD, Small DS. Nonparametric methods for doubly robust estimation of continuous treatment effects. Philos Trans R Soc B. 2017;79(4):1229–45.
134. Cole SR, Frangakis CE. Commentary: the consistency statement in causal inference: a definition or an assumption? Epidemiology. 2009;20(1):3–5.
135. Hernan MA, Taubman SL. Does obesity shorten life? The importance of well-defined interventions to answer causal questions. Int J Obes. 2008;32(Suppl 3):S8–14.
136. Weisskopf MG, Webster TF. Trade-offs of personal versus more proxy exposure measures in environmental epidemiology. Epidemiology. 2017;28(5):635–43.
137. Rehkopf DH, Glymour MM, Osypuk TL. The consistency assumption for causal inference in social epidemiology: when a rose is not a rose. Curr Epidemiol Rep. 2016;3(1):63–71.
138. Pearl J. On the consistency rule in causal inference: axiom, definition, assumption, or theorem? Epidemiology. 2010;21(6):872–5.
139. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet. 2014;23(R1):R89–98.
140. Pierce BL, Tong L, Argos M, Gao J, Jasmine F, Roy S, et al. Arsenic metabolism efficiency has a causal role in arsenic toxicity: Mendelian randomization and gene-environment interaction. Int J Epidemiol. 2014;42(6):1862–72.
141. Cedeño Laurent JG, Williams A, Oulhote Y, Zanobetti A, Allen JG, Spengler JD. Reduced cognitive function during a heat wave among residents of non-air-conditioned buildings: an observational study of young adults in the summer of 2016. PLoS Med. 2018;15(7):e1002605.
142. Kelly C. Bishop JDK, Nicolai V. Kuminoff. Hazed and confused: the effect of air pollution on dementia. NBER Working Paper No 24970. 2018.
143. Nassan FL, Coull BA, Skakkebaek NE, Williams MA, Dadd R, Mínguez-Alarcón L, et al. A crossover–crossback prospective study of dibutyl-phthalate exposure from mesalamine medications and semen quality in men with inflammatory bowel disease. Environ Int. 2016;95:120–30.
144. Philippat C, Botton J, Calafat AM, Ye X, Charles M-A, Slama R, et al. Prenatal exposure to phenols and growth in boys. Epidemiology. 2014;25(5):625–35.
145. MacLehose RF, Olshan AF, Herring AH, Honein MA, Shaw GM, Romitti PA, et al. Bayesian methods for correcting misclassification: an example from birth defects epidemiology. Epidemiology. 2009;20(1):27–35.
146. Oulhote Y, Steuerwald U, Debes F, Weihe P, Grandjean P. Behavioral difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances. Environ Int. 2016;97:237–45.

147. Schmidt RJ, Kogan V, Shelton JF, Delwiche L, Hansen RL, Ozonoff S, et al. Combined prenatal pesticide exposure and folic acid intake in relation to autism spectrum disorder. Environ Health Perspect. 2017;125(9):097007.
148. Goodrich AJ, Volk HE, Tancredi DJ, McConnell R, Lurmann FW, Hansen RL, et al. Joint effects of prenatal air pollutant exposure and maternal folic acid supplementation on risk of autism spectrum disorder. Autism Res. 2018;11(1):69–80.
149. Lemire M, Fillion M, Frenette B, Mayer A, Philibert A, Passos CJ, et al. Selenium and mercury in the Brazilian Amazon: opposing influences on age-related cataracts. Environ Health Perspect. 2010;118(11):1584–9.
150. McCoy DC, Peet ED, Ezzati M, Danaei G, Black MM, Sudfeld CR, et al. Early childhood developmental status in low- and middle-income countries: national, regional, and global prevalence estimates using predictive modeling. PLoS Med. 2016;13(6):e1002034.

Chapter 8
Immunotoxicity: Impacts and Research Approaches

Carsten Heilmann and Philippe Grandjean

Abstract The immune system is vulnerable to toxicant exposures during early development, but environmental chemicals have only recently been considered as possible immunotoxicants. So far, immunotoxicity is not routinely considered in the risk assessment of industrial chemicals, even less so developmental exposures, despite evidence on dioxins and related compounds, certain metals, and recent information on the perfluorinated alkylate substances (PFASs). The mouse is an especially useful experimental model. Recent epidemiological studies have employed the antibody response to childhood immunizations as a clinically relevant outcome that reflects major immune system functions. Lowered antibody responses occur along with increased frequency of infectious disease in children at elevated PFAS exposure, including early postnatal exposure that may affect B cell maturation. In addition, autoimmune disease, such as ulcerous colitis, is a possible outcome that requires further study, as does immune suppression in regard to possible carcinogenicity. Beyond the PFASs and other known immunotoxicants, most environmental chemicals have not been properly tested and may well represent immunotoxic potentials. Given the public health impact from existing and emerging infectious diseases and other potential consequences, developmental immunotoxicity should no longer be ignored as an important adverse effect of environmental chemicals.

Keywords Antibodies · Developmental toxicity · Immune functions · Immunity · Humoral · Perfluorinated compounds · Prospective study · Vaccination

C. Heilmann
Paediatric Clinic II, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
e-mail: Carsten.Heilmann@regionh.dk

P. Grandjean (✉)
Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

Department of Environmental Medicine, University of Southern Denmark, Odense, Denmark
e-mail: pgrand@hsph.harvard.edu

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_8

The immune system plays a crucial role in higher animals as it distinguishes self from non-self to allow proper responses to threats. While the immune system is necessary for the defense of each individual against infectious agents, it also has a broader impact by enabling an entire population to maintain health and survival. Thus, to protect against zoonoses and infestations, public health efforts have a dual purpose, i.e., to control the environmental sources of pathogens and to maintain individual resistance toward infection and infestation. This chapter will focus on the role of the immune system to protect against communicable disease and other disease risks and how chemical exposures during early development may affect the resistance against pathogens, allergens, and carcinogens.

Our immune functions are in no way stable and predictable, but are sensitive to a variety of environmental factors. For example, malnutrition and vitamin-A deficiency [1] may threaten an individual's immune function and result in augmented susceptibility to infectious disease but may also allow otherwise low-virulent pathogens to spread faster and potentially cause deleterious effects, as has been observed for tuberculosis, malaria, or influenza. Adverse effects on immune functions first emerged as an important safety concern regarding drugs [2]. Later on, the question arose whether environmental chemicals could interfere with immune system development and functions, whether in the form of immunosuppression, immunostimulation, hypersensitivity, or autoimmunity [3–5]. Only in recent years has research begun to focus on developmental immunotoxicity [6].

Recognized environmental immunotoxicants include dioxins, polychlorinated biphenyls (PCBs) [6], perfluorinated alkylate substances (PFASs) [7], mercury [8, 9], lead [10], and certain pesticides [11, 12]. Some of these substances are also considered endocrine disrupters, and experimental studies using diethylstilbestrol have clearly shown the potential link to immunotoxicity [13]. Still, all of these substances differ substantially from one another, and they likely affect different targets of complex immune functions [6, 14]. Thus, it is by no means clear at this time if specific immune functions are particularly vulnerable to specific toxicants and to which degree susceptibility is increased during early development of immune functions.

In contrast to other organ-related toxicity, even minor immunosuppression, at an individual level, may appear subtle but could allow invasion of a contagious pathogen, and even a mild infection could represent an important impact on disease dissemination on a population basis [15, 16]. Furthermore, even modest dysregulation may result in adverse effects in individuals with pre-existing conditions, such as AIDS, cancer, organ transplantation, allergies, autoimmune diseases, and old age. However, our understanding of these processes is limited, and the health impact of environmental immunotoxicity is probably grossly underestimated [2, 17]. This chapter will briefly outline some main properties of the immune system, its development, methods for assessing important immune system functions, and approaches to human studies, with an emphasis of vaccine responses as an important outcome in epidemiology research.

8.1 Immune System Functions and Dysfunctions

Due to the crucial importance of the immune system, multiple lines of defense have evolved, so that a temporary breakdown of one line of defense in an individual can be compensated by other available mechanisms. This complex system becomes functional only as a result of a multifaceted development that begins with pluripotent hematopoietic cells that differentiate into a wide range of cells with different and interconnected functions. Because the adverse effects caused by environmental immunotoxicants are poorly understood [6, 14], developmental immunotoxicology is a research field that is likely to burgeon.

The immune system is normally divided into two main categories: The innate immune system and the adaptive immune system, although the two systems collaborate [14]. The innate immune system is the phylogenetically older system and is in many ways more important for maintaining continuous basic immunity than the adaptive immune system. The more advanced and phylogenetic younger adaptive system in vertebrates first comes into play when the innate system has failed. The innate immune system consists of many very different elements, such as white blood cell populations of neutrophils and macrophages, but also important soluble factors, such as complement, C-reactive protein (CRP), and mannose-binding lectins, as well as membrane bound components (e.g., in ciliated epithelium, goblet cells, and gastric acid producing cells). The main elements are generated during prenatal development and form the basis of protection from the time that the infant enters the outside world.

The adaptive immune system relies on functions developed prenatally, but must mature in response to external stimuli that it is confronted with after entering the outside world. The immune system needs to generate memory and increased affinity to unwanted exogenous stimuli upon repeated stimulation. For this purpose, it includes two lymphocyte lines, the so-called T lymphocytes and the B lymphocytes. The T cells can be subdivided into many different subpopulations (e.g., T helper cells, T regulatory cells, T cytotoxic cells) based on their function, surface markers, and their production and secretion of cytokines. T helper cells may be further subdivided into, e.g., T1 helper, T2 helper, and T17 helper cells. Defined by their characteristic cytokine production profile, T1 helper cells predominantly seem to help cytotoxic cells to combat viral and fungus infections whereas T2 helper cells play an important role for production of antibody and for type I allergic reactions. In contrast, T17 helper cells are proinflammatory and of importance for the defense against fungus. T cells may also be subdivided according to their maturity and whether or not they are descendants of antigen-activated cells (recent thymic emigrants (RTE), T-RA (naïve), T-RO (memory)).

The B cells are regulated by the T helper cells, and these cells are responsible for the production of immunoglobulin G (IgG) antibodies as well as other Ig classes (in humans, IgM, IgD, IgG1-4, IgA1-2, and IgE). B cells may, upon stimulation, continue their development into plasma cells which are low in membrane bound molecules and receptors, but function as secretory cells of Ig with a long life capability.

Major breakdown of the innate immune system can cause the immediate death of an individual (if not intensively treated), as can be observed in relation with diseases, such as aplastic anemia and meningococcal septicemia. Still, the absence of adaptive immunity may allow survival for some time, as seen in relation with severe combined immunodeficiency, an inborn error of immunity where antibodies transferred from the mother provide initial protection.

Due to the vital role of the immune system to maintain health, a major research goal is to measure quantitatively the different lines of defense within both the innate and the adaptive immune systems. However, many immune functions cannot be measured quantitatively, but only indirectly. Thus, proper immune function relies on many different and interacting factors and functions, and there is no simple relation between quantity and quality, and reservations will always apply when interpreting, e.g., antibody concentrations in regard to individual health.

To assess immune functions and the impact of immunotoxicants, it is often necessary to rely on in vitro and/or laboratory animal studies, where the mouse has turned out to be a reliable model. However, account must be taken of species-related differences, e.g., in the length of gestation or in the production of immunoglobulin (Ig) types. As studies of human volunteers under controlled conditions would be useful, they must necessarily focus on beneficial effects of immune function changes. Epidemiological immunotoxicity studies can benefit from occupational health surveillance, but characterization of developmental effects in humans usually requires "natural" experiments that result in case reports or allow prospective studies of birth cohorts.

8.2 Evaluation of Immunotoxicity

The exploration of immune functions in laboratory animals exposed to new therapeutic agents or potential toxicants is well established, although still limited in scope [15]. While substantial experimental evidence does exist on the impact of adverse factors on immune functions in animal models, systematic studies on immunotoxicity due to industrial chemicals have not been carried out. The OECD has developed a guideline for assessment of immunotoxicity in rodent models, and so has the US EPA (OPPTS 870.7800), but both refer to adult animals only [18]. In addition to the lack of standard procedures, a main issue is that immunotoxicity has not yet been considered an important outcome in the assessment of chemical safety. Thus, research in this field is still in an early phase of exploration, and both plausibility of new findings and their public health relevance can be difficult to evaluate. Quite likely, this lack of documentation results in insufficient protection of public health.

The lack of recommended procedures is likely due to the difficulties in standardizing assessment of complex immune system development, where minute differences can have profound effects, and where interspecies extrapolation may be problematic. The wide variety of experimental approaches also impedes the

comparability of results. Although the evidence available is far from systematic, studies of developmental immunotoxicity often measure thymus, spleen, and liver size or weight as well as leucocyte differential counts. Experimental studies may also explore survival, e.g., after influenza A infection [19]. More rarely, functional studies are employed (e.g., antibody formation, lymphocyte function tests), sometimes after developmental exposure [7]. However, these experimental data on immune functions can be difficult to interpret due to their uncertain human relevance, where the concern is usually the low-level mixed toxicant exposures at different developmental stages [14].

As an example, it is plausible that the activation of the aryl hydrocarbon receptor (AhR) by exposure to dioxin and certain PCBs may affect the thymus function. Thus, activation of the murine AhR by dioxin dosage can influence the differentiation of proinflammatory interleukin (IL) 17 producing T helper lymphocytes and T regulatory cells [20]. Especially the so-called coplanar PCBs may cause a dioxin-like immune suppression by binding to the AhR receptor (aryl hydrocarbon receptor) [21]. However, the non-coplanar PCBs also may have immune depressant properties [22]. Support from prospective studies in children refers to diminished thymus size [23], and to reduced vaccination response and increased frequency of infections [24–26]. However, the extent to which AhR interaction can explain these associations is unclear. Nonetheless, the absence of a known adverse outcome pathway should not be misunderstood to undermine the findings of human PCB immunotoxicity.

A combination of in vitro and in vivo studies is useful to explore modes of action [14], as has been done in regard to the PFASs [27–30]. Following our discovery that early-life exposure to PFASs can lower the antibody response to vaccines [31], we examined in vitro human immunotoxicity by perfluorinated octane sulfonate (PFOS) and perfluorinated octanoic acid (PFOA). We simulated human B lymphocyte activation by T lymphocytes in lymph nodes by adding a CD40 ligand to peripheral blood mononuclear cells for 10 min and examined their ability to up-regulate the signal molecule NF-κB in the B lymphocytes by flow cytometry after staining the cells with anti-CD19-PE and anti-NF-κB FITC (Fig. 8.1). These results show diminished NF-KB up-regulation if exposed to at least 10 μg/mL of the two PFASs. Although these concentrations are much higher than serum levels, the results suggest a possible mode of action.

Among useful experimental approaches, antibody formation, e.g., in $B6C_3F_1$ mice after antigen stimulation has turned out to be a feasible and promising assay, often using injection of sheep erythrocytes as a standard antigen stimulus [14]. A decreased antibody response occurs after exposure to environmentally relevant doses of PFASs [32]. This design is particularly relevant to immunotoxicity assessment, because antibody formation is the product of a broad combination of relevant functions central to adaptive immunity. Hence, B-cell priming and antibody formation constitute the result of an intriguing interplay between antigen presentation by cells, such as macrophages, histiocytic cells or dendritic cells, with Th cells and later on Th cells with B cells. This complex process stimulates B cells to produce IgM antibody, which is followed—upon prolonged or repeated exposure to the

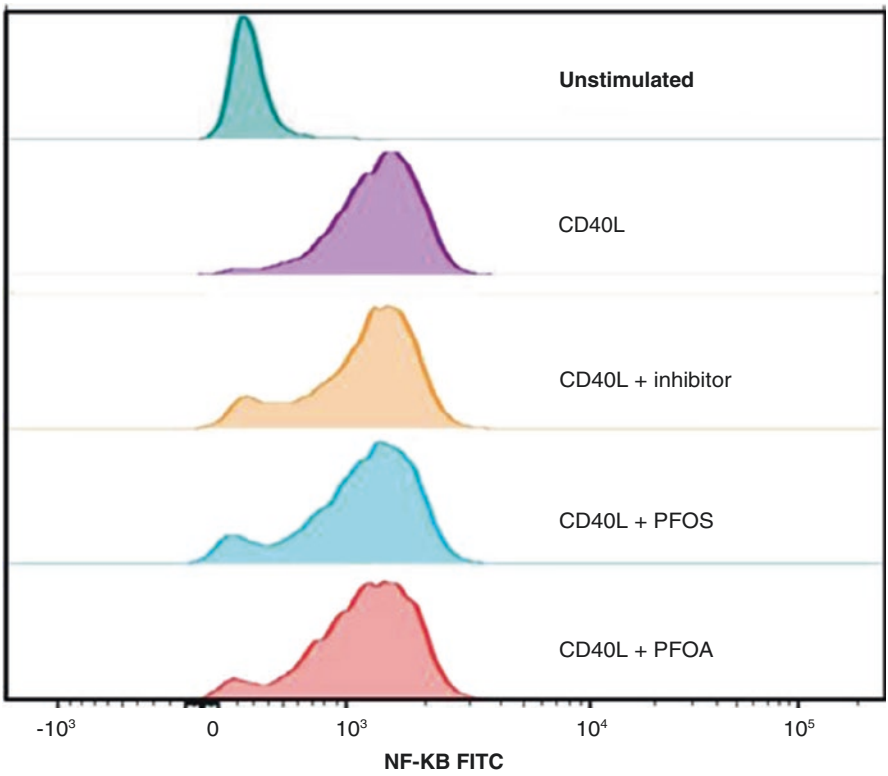


Fig. 8.1 To simulate in vitro the stimulation of B lymphocytes by T lymphocytes in lymph nodes CD40 ligand was added to peripheral blood mononuclear cells for 10 min and the ability to up-regulate NF-KB in B lymphocytes was recorded by flow cytometry

antigen—by Ig switching to other Ig classes (IgG) and to maturation (improvement) of the antibody affinity. The attractive characteristic is that the outcome of this process, the antibody concentration in serum, can be measured by sensitive and reproducible assays in laboratory animals as well as in humans.

8.3 Vaccine Types and Immunization Processes

The use of antibody concentrations as a relevant outcome parameter was suggested already at a research symposium in 1999 [8] and was later adapted by recommendations issued by the World Health Organization [33, 34]. According to these conclusions, human immune functions could be appropriately evaluated by taking advance of the priming of the immune system by routine vaccines in children who have not been previously exposed to the particular antigen in question.

As randomized clinical trials are not possible when dealing with immunotoxicants that are not used for therapeutic purposes, most evidence on adverse effects on human immune functions are based on observational studies, whether cross-sectional or prospective. This evidence must take into account individual variations in immune competence, uncertainties in assessing past and current exposures, impacts of other risk factors, and the methodological concerns regarding sample conditions from the time of blood sampling to the assay in the laboratory. Thus, robustness of the immunological tests chosen can be a serious concern in human population studies. In this regard, assessment of specific IgG antibodies against vaccine antigens is advantageous due to their stability and the wide availability of standardized ELISA assays.

In industrialized countries, infants and small children are routinely vaccinated against bacterial diseases such as diphtheria, tetanus, whooping cough, as well as diseases caused by certain types of pneumococci (Pn) and *Haemophilus influenzae* type b (Hib). Furthermore, children are immunized against viral diseases such as measles, mumps and rubella (MMR) as well as polio (and in some countries also varicella). Overall, the World Health Organization estimates that vaccinations globally saves 2 million lives every year (https://www.who.int/publications/10-year-review/vaccines/en/) and the Centers for Disease Control considers vaccinations one of the cornerstones of modern prevention [35]. Thus, maintenance of desirable vaccination responses is a public health priority, and individual vaccination responses also constitute a promising indicator of immune functions and the possible impact of adverse stimuli, as illustrated by studies of childhood exposures to PCBs and dioxins [24, 26, 36, 37].

Vaccines differ in many ways, not only with respect to the organism they protect against, but also how the immune system processes antigens of different origins [38]. Some vaccines are live attenuated organisms (e.g., MMR and varicella and in some countries also polio in the form of OPV), and they particularly give rise to T-cell immunity that is more difficult to quantify.

Other vaccines constitute chemical components from the individual microorganism (Pn, Hib, *Bordetella pertussis*) or toxoids from bacteria, such as diphtheria toxoid (DT) and tetanus toxoid (TT). These vaccines—the so-called dead vaccines—primarily give protection by inducing specific antibody production. For some vaccines, the antibody level induced by immunization has a reasonable correlation with the degree of protection, which is the case for Hib, Pn, DT, and TT. However, for the live vaccines, the mere finding of a positive titer is a surrogate indicator for protection by T cells.

Some vaccines contain true de novo priming antigens (DT, TT, MMR, VZV) whereas others may in some cases cross-react with microorganisms normally encountered by the child (Hib, Pn). Thus, Hib cross-reacts with certain *E. coli* strains and perhaps also certain food substances. Some Pn strains are encountered in early life, especially in infants with older siblings or infants cared for in kindergartens. Accordingly, antibodies against Hib and Pn types may well occur in children who have not been vaccinated.

An additional aspect of importance, some antigens are peptides or proteins (DT, TT), whereas others are polysaccharides that most often must be conjugated to a protein carrier to make the antigen accessible to T cell help (Pn and Hib vaccines) [39]. Thus, the way that B-cell stimulation is elicited is markedly different between polysaccharides and protein/peptides. B cells that recognize proteins depend on T helper cells cross-talking with a protein-derived peptide nested in the HLA class 2 molecule of the B cell. This is the case both for priming of B cells and secondary stimulation (so-called T-cell dependent stimulation). In contrast, re-stimulation of secondary B lymphocytes with specificity for polysaccharides may be achieved by the polysaccharide alone (so-called T-cell independent stimulation). These differences are crucial to take into account when designing immunotoxicity studies and evaluating study outcomes.

8.4 Perfluorinated Alkylate Substances (PFASs)

Early toxicology studies on PFOA were commissioned by a major producer and were briefly summarized in 1980 [40]; the liver was highlighted as a target organ, while effects on the immune (or reticuloendothelial) system were also reported. Details, however, were not provided, but the study report was eventually submitted to the US Environmental Protection Agency in 2000. This information was unknown to the European Food Safety Authority in 2008 [41], which did not consider immunotoxicity as a relevant outcome from exposures to PFOS and PFOA. Thus, apart from an early rodent study of decreased antibody formation against sheep red blood cells and spleen enlargement [42], little was published on PFAS immunotoxicity until about 10 or 12 years ago, where the effects on antibody formation were confirmed at serum-PFAS concentrations relevant to human exposures [43]. Additionally, one mouse study demonstrated reduced survival after influenza A infection [19], and another showed that male pups after gestational exposure were more sensitive than females to PFOS immunotoxicity and that functional deficits in innate and humoral immunity remained detectable at adult age [32].

We chose to focus on vaccination responses as a key immunotoxicity outcome of public health relevance and of great attraction in regard to its feasibility in epidemiological studies. As a proof of concept, we vaccinated adults—who had all received routine vaccinations in the past—against tetanus and diphtheria and followed the antibody production by analysis of serial blood samples during the month after the inoculation [44]. Some of the 12 subjects barely responded to the vaccination, and the steepness of the response decreased at higher serum-PFAS concentrations. Similarly, a study of over 400 adults showed that higher serum-PFOA concentrations showed decrease response to a flu (A/H3N2) vaccination [45].

In a prospective birth cohort of 656 births in the Faroe Islands, 587 of the children were followed through to ages 5 and 7 years. A doubling of the exposure to PFOS and PFOA was associated with an overall decrease by about 50% in the antibody concentration (Fig. 8.2) [31, 46]. These results suggest an influence on T cells

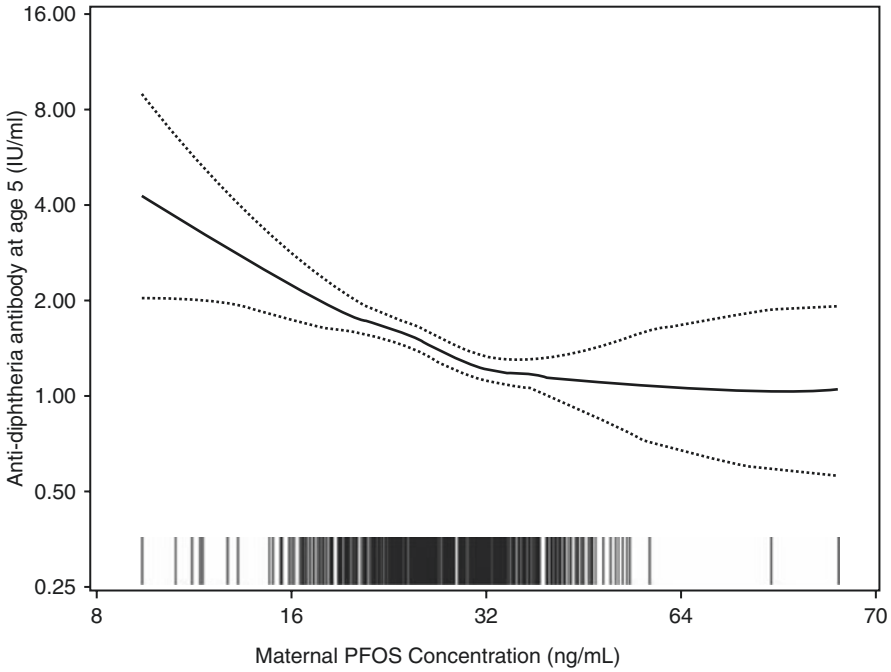


Fig. 8.2 Generalized additive model (with 95% confidence interval) showing diphtheria Ab concentrations at age 5 years associated with PFOS concentrations in the mother's pregnancy serum [31]

by PFASs. Thus, we have found that the antibody-increase following booster vaccination at age 5 years with DT and TT was more rapid in individuals with low serum-PFAS concentrations than in those with high concentrations, thereby suggesting a diminished T cell help in the latter group.

As a further concern, a substantial number of children at age 7 had such a low antibody concentration that they had no long-term protection against the targeted diseases despite a total of four vaccinations. For doubled concentrations at age 5, PFOS and PFOA showed odds ratios (ORs) between 2.4 and 4.2 for falling below the clinically protective antibody level [31, 47]. Adjustment for elevated PCB exposure did not materially affect the calculations, as would be expected due to the poor correlation between the two [31]. The incomplete protection by a complete series of immunizations would seem to be of public health relevance.

We also examined antibody concentrations against Pn and HiB following booster vaccination with the conjugated 13 valent Pn vaccine and the conjugated Hib vaccine but did not find correlation with exposures to PCB or PFASs (Grandjean and Heilmann, unpublished results). This difference between the protein antigens (DT and TT) and the polysaccharide antigens (Pn and Hib) may be related to the former being T dependent and the latter being at least partly T-independent. Thus, the polysaccharide antigens do not require T-cell help for the secondary immunizations

(boosting), whereas the proteins do. This observation suggests that toxicity from PCBs and PFASs affects T cell helper functions rather than to a direct effect on B cells. Of further concern, antigens such as Pn and Hib are not necessarily de novo antigens. Some infants/children may, as mentioned earlier, encounter similar (cross-) reacting antigens and thus influence the antibody levels and in this way blur the results.

The findings of lowered concentrations of vaccine antibodies at elevated PFAS exposures are consistent with a smaller study of 3-year-old children, where inverse correlations were found between the mother's serum-PFAS concentrations during early pregnancy and decreased antibody levels in their children against four different childhood vaccinations [48]. This study also found that increased PFOA concentrations were linked to substantial increases in the incidence of the children suffering from the common cold and gastroenteritis. Thus, elevated PFAS exposures were linked to both lower antibody concentrations and more frequent infections.

As the adaptive immune system is programmed during early development, immunotoxicity assessment is particularly relevant in subjects with PFAS exposures during early life [49]. PFASs are excreted in human milk, and breastfed children may thus be particularly at risk [50]. Thus, in a more recent Faroese birth cohort, we estimated serum-PFAS concentration profiles during infancy based on the duration of breastfeeding, and the calculations were validated by comparison with measured serum-PFAS concentrations at age 18 months. Again, inverse associations between prenatal exposure levels and age-5 serum concentrations of antibodies against tetanus and diphtheria vaccines were observed. Further estimated serum concentrations at ages 3 and 6 months also showed strong inverse associations with antibody concentrations at age 5 years, i.e., more than 4 years later, particularly for tetanus (Table 8.1). These associations were stronger than those seen for PFAS concentrations at ages 18 months and 5 years and therefore support the notion that the developing adaptive immune system is particularly vulnerable to immunotoxic exposures, e.g., to PFOA, during infancy. This finding also means that studies relying on serum concentrations that do not reflect ages at peak vulnerability will likely underestimate the true effects.

Table 8.1 Change (in %) of tetanus and diphtheria Ab concentration at age 5 years associated with a doubling of the calculated serum-PFOA concentration at three infancy ages (with 95% confidence intervals in parenthesis)

	Tetanus		Diphtheria	
Age (months)	Change	95% CI	Change	95% CI
0[a]	−22.3	−35.3, −6.6	−18.9	−33.2. −1.7
3	−32.6	−46.7, −14.8	−12.4	−30.6, 10.7
6	−24.8	−38.4, −8.2	−6.4	−23.3, 14.1
12	−16.9	−30.1, −1.3	−3.9	−19.0, 14.0
18[a]	−16.3	−29.0, −1.3	4.2	−11.8, 23.0
60[a]	−25.3	−42.6, −2.6	18.3	−10.7, 56.8

[a]Results based on measured PFOA concentrations in maternal pregnancy and child serum [49]

8.5 Other Outcomes

Relevant immune system outcomes regard the adverse impacts on the body's ability to fight off various common diseases including colds, fevers, and gastroenteritis, as already mentioned [48]. Thus, a study of Danish children from the Odense Child Cohort followed 359 children at ages 1–3 years by monitoring the frequency of fever and associated symptoms every 2 weeks for a year (via text messages). Increased maternal serum concentrations of PFOA and PFOS were significantly associated with a higher frequency of fever, also in combination with nasal discharge or cough [51]. However, another Danish cohort study examined the frequency of hospitalizations for infection of the children through to age 11 years and failed to identify any association with maternal serum concentrations of PFOS and PFOA [52]. However, the study merged frequencies of diagnoses, such as airway infection, appendicitis, middle ear infection, and a recent report raised doubt about the validity of the blood analyses [53].

In contrast, a Norwegian study showed that prenatal exposure to PFASs was associated with the frequency of respiratory tract infections during the first 10 years of life [54], and Japanese findings show a similar tendency in 4-years old children [55]. These findings strengthen the already existing evidence for PFAS immunotoxicity and its clinical impact.

On a population scale, infectious disease will spread as long as a sufficient number of individuals are susceptible and not protected from past infection or immunization. Immunotoxicity can therefore weaken the herd immunity (also called community immunity), where pervasiveness of the immunity in the population can prevent transmission of the infectious agent [56]. Thus, a relatively small impact on individual health may have serious consequences for the health at the population level. So far, this remains to be addressed in studies of immunotoxicants.

PFASs also have been found to be linked to certain forms of autoimmune disease, in which the body's immune system attacks its own tissues. This link is demonstrated by an occupational health study of 3713 workers, whose PFOA exposures were assessed. Using a 10-year lag, the occurrence of ulcerative colitis and, without a lag, rheumatoid arthritis showed significant associations by greater disease frequencies at elevated PFOA exposures [57]. Another study concerned a general population exposed to PFOSA from contaminated drinking water, where 151 cases of ulcerous colitis were identified in connection with the medical examinations. With a p value less than 0.0001, higher serum-PFOA concentrations predicted a greater risk of developing the disease [58]. In a recent cross-sectional US study, young ulcerous colitis patients had higher serum concentrations of PFOA than controls and those with Crohn's disease [59], but the serum samples were collected within a year of diagnosis, not before.

Allergies may also be related to PFAS immunotoxicity, as reported by studies linking PFAS exposure to increased development of allergies in children [60]. Thus, a study of the Faroese birth cohort born in 1997–2000 included data on allergy and asthma at ages 5 to 13 years [61]. Twenty-two of the 559 children had

not been vaccinated against MMR, and among those, higher serum concentrations of the five PFASs at age 5 years (but not prenatally) were associated with increased odds of asthma at ages 5 and 13. However, the associations were reversed among MMR-vaccinated children, suggesting that MMR vaccination might be an effect modifier.

To further explore the mechanisms, a study was carried out in Norway to characterize gene expression in cord blood and its association with PFAS concentrations, antibody concentrations, and infectious disease incidence. Several immunomodulatory genes, especially the C17 gene, were linked to all three parameters, and these findings therefore supported a PFAS-linked genetic mechanism underlying both the lowered antibody response and the increased susceptibility to infectious disease [62]. While other studies have examined allergy and asthma frequencies in relation to PFAS exposures, the variable findings may be due to differences in allergen exposures at different ages as well as assessment methods and exposure measures.

8.6 Conclusions

Immunotoxicity is not routinely considered in risk assessment of environmental chemicals, even less so in regard to developmental exposures. The experience obtained on the PFASs as global pollutants is illustrative. Although these compounds have been disseminated in the environment for several decades, their immunotoxic potential was not appreciated until about 10 or 12 years ago. A variety of experimental studies demonstrated immunotoxicity, also from developmental exposures, and recent epidemiological studies have employed the antibody response to childhood immunizations as a clinically relevant outcome that reflects major immune system functions. Thus, study subjects have all received the same doses of antigen (in the form of the vaccines) at the same ages, and examinations can then be scheduled at similar ages, i.e., at similar intervals after the most recent vaccination. Recent evidence suggests that both prenatal exposures and those incurred early postnatally are associated with diminished antibody responses. The US National Toxicology Program concluded in 2016, on the basis of both experimental and epidemiological studies, that PFOA and PFOS are likely, or "presumed to be," human immunotoxicants [63]. The NTP uses the term "presumed" to denote the level of evidence just below "known," and stronger than "suspected." In addition, autoimmunity, including ulcerous colitis, was considered as a possible outcome, although with "low confidence" according to the NTP, due to the few studies available. These findings suggest that other environmental chemicals that have been examined to an even lesser degree than the PFASs may also have immunotoxic potentials. Given the public health impact from existing and emerging infectious diseases [64], immunotoxicity should no longer be ignored as an important adverse effect of environmental chemicals.

References

1. Semba RD. Vitamin A as "anti-infective" therapy, 1920–1940. J Nutr. 1999;129(4):783–91.
2. Descotes J. Immunotoxicology: role in the safety assessment of drugs. Drug Saf. 2005;28(2):127–36.
3. Gleichmann E, Kimber I, Purchase IF. Immunotoxicology: suppressive and stimulatory effects of drugs and environmental chemicals on the immune system. A discussion. Arch Toxicol. 1989;63(4):257–73.
4. Van Loveren H, Steerenberg PA, Vos JG. Early detection of immunotoxicity: from animal studies to human biomonitoring. Toxicol Lett. 1995;77(1–3):73–80.
5. Ilback NG, Friman G. Interactions among infections, nutrients and xenobiotics. Crit Rev Food Sci Nutr. 2007;47(5):499–519.
6. Winans B, Humble MC, Lawrence BP. Environmental toxicants and the developing immune system: a missing link in the global battle against infectious disease? Reprod Toxicol. 2011;31(3):327–36.
7. DeWitt JC, Blossom SJ, Schaider LA. Exposure to per-fluoroalkyl and polyfluoroalkyl substances leads to immunotoxicity: epidemiological and toxicological evidence. J Expo Sci Environ Epidemiol. 2019;29(2):148–56.
8. Sweet LI, Zelikoff JT. Toxicology and immunotoxicology of mercury: a comparative review in fish and humans. J Toxicol Environ Health B Crit Rev. 2001;4(2):161–205.
9. Oulhote Y, Shamim Z, Kielsen K, Weihe P, Grandjean P, Ryder LP, et al. Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. Reprod Toxicol. 2017;68:207–14.
10. Dietert RR, Lee JE, Hussain I, Piepenbrink M. Developmental immunotoxicology of lead. Toxicol Appl Pharmacol. 2004;198(2):86–94.
11. Karmaus W, Kuehr J, Kruse H. Infections and atopic disorders in childhood and organochlorine exposure. Arch Environ Health. 2001;56(6):485–92.
12. Vine MF, Stein L, Weigle K, Schroeder J, Degnan D, Tse CK, et al. Plasma 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) levels and immune response. Am J Epidemiol. 2001;153(1):53–63.
13. Luster MI, Hayes HT, Korach K, Tucker AN, Dean JH, Greenlee WF, et al. Estrogen immunosuppression is regulated through estrogenic responses in the thymus. J Immunol. 1984;133(1):110–6.
14. Dietert RR. Immunotoxicity testing: methods and protocols. New York: Humana Press; 2010. p. 430.
15. Heilmann C. Immunotoxicity, immune dysfunction, and chronic disease. Infections and cancer. In: Luebke RW, Dietert RR, editors. Environmental toxicants and susceptibility to infection. New York: Humana Press; 2012. p. 389–98.
16. Feingold BJ, Vegosen L, Davis M, Leibler J, Peterson A, Silbergeld EK. A niche for infectious disease in environmental health: rethinking the toxicological paradigm. Environ Health Perspect. 2010;118(8):1165–72.
17. DeWitt JC, Peden-Adams MM, Keil DE, Dietert RR. Current status of developmental immunotoxicity: early-life patterns and testing. Toxicol Pathol. 2012;40(2):230–6.
18. Agency USEP. Health effects test guidelines. OPPTS 870.7800 immunotoxicity. Washington: US Environmental Protection Agency; 1998. Report No.: Publication EPA 712-C-98-351.
19. Guruge KS, Hikono H, Shimada N, Murakami K, Hasegawa J, Yeung LW, et al. Effect of perfluorooctane sulfonate (PFOS) on influenza A virus-induced mortality in female B6C3F1 mice. J Toxicol Sci. 2009;34(6):687–91.
20. Quintana FJ, Basso AS, Iglesias AH, Korn T, Farez MF, Bettelli E, et al. Control of T(reg) and T(H)17 cell differentiation by the aryl hydrocarbon receptor. Nature. 2008;453(7191):65–71.
21. European Food Safety Authority. Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in feed and food. EFSA J. 2018;16(11):1–331.

22. Levin M, Morsey B, Mori C, Nambiar PR, De Guise S. Non-coplanar PCB-mediated modulation of human leukocyte phagocytosis: a new mechanism for immunotoxicity. J Toxicol Environ Health A. 2005;68(22):1977–93.
23. Park HY, Hertz-Picciotto I, Petrik J, Palkovicova L, Kocan A, Trnovec T. Prenatal PCB exposure and thymus size at birth in neonates in Eastern Slovakia. Environ Health Perspect. 2008;116(1):104–9.
24. Stolevik SB, Nygaard UC, Namork E, Haugen M, Meltzer HM, Alexander J, et al. Prenatal exposure to polychlorinated biphenyls and dioxins from the maternal diet may be associated with immunosuppressive effects that persist into early childhood. Food Chem Toxicol. 2013;51:165–72.
25. Weisglas-Kuperus N, Vreugdenhil HJ, Mulder PG. Immunological effects of environmental exposure to polychlorinated biphenyls and dioxins in Dutch school children. Toxicol Lett. 2004;149(1–3):281–5.
26. Heilmann C, Budtz-Jorgensen E, Nielsen F, Heinzow B, Weihe P, Grandjean P. Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to immunotoxicants. Environ Health Perspect. 2010;118(10):1434–8.
27. Corsini E, Avogadro A, Galbiati V, dell'Agli M, Marinovich M, Galli CL, et al. In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCs). Toxicol Appl Pharmacol. 2011;250(2):108–16.
28. Corsini E, Sangiovanni E, Avogadro A, Galbiati V, Viviani B, Marinovich M, et al. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCs). Toxicol Appl Pharmacol. 2012;258(2):248–55.
29. Dong GH, Zhang YH, Zheng L, Liu W, Jin YH, He QC. Chronic effects of perfluorooctanesulfonate exposure on immunotoxicity in adult male C57BL/6 mice. Arch Toxicol. 2009;83(9):805–15.
30. Dong GH, Liu MM, Wang D, Zheng L, Liang ZF, Jin YH. Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. Arch Toxicol. 2011;85(10):1235–44.
31. Grandjean P, Andersen EW, Budtz-Jorgensen E, Nielsen F, Molbak K, Weihe P, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA. 2012;307(4):391–7.
32. Keil DE, Mehlmann T, Butterworth L, Peden-Adams MM. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. Toxicol Sci. 2008;103(1):77–85.
33. International Programme for Chemical Safety. Environmental health criteria 180: principles and methods for assessing direct immunotoxicity associated with exposure to chemicals. Geneva: World Health Organization; 1996.
34. World Health Organization. Guidance for immunotoxicity risk assessment for chemicals: IPCS harmonization project. Geneva: WHO; 2012. Contract No.: 10.
35. Center for Disease Control and Prevention (CDC). Ten great public health achievements: United States, 1900–1999. Morb Mortal Wkly Rep. 1999;48:241–3.
36. Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, et al. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. Environ Health Perspect. 2000;108(12):1203–7.
37. Heilmann C, Grandjean P, Weihe P, Nielsen F, Budtz-Jorgensen E. Reduced antibody responses to vaccinations in children exposed to polychlorinated biphenyls. PLoS Med. 2006;3(8):e311.
38. Plotkin S, Orenstein W, Offit P, Edwards KM. Plotkin's vaccines, vol. 7. Philadelphia: Elsevier; 2017.
39. Heilmann C. Human B and T lymphocyte responses to vaccination with pneumococcal polysaccharides. APMIS Suppl. 1990;15:1–23.
40. Griffith FD, Long JE. Animal toxicity studies with ammonium perfluorooctanoate. Am Ind Hyg Assoc J. 1980;41(8):576–83.
41. European Food Safety Authority. Opinion of the scientific panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. EFSA J. 2008;653:1–131.

42. Bollands AD, Lowe KC. Effects of a perfluorocarbon emulsion, Fluosol-DA, on rat lymphoid tissue and immunological competence. Comp Biochem Physiol C. 1986;85(2):309–12.
43. Peden-Adams MM, Keller JM, Eudaly JG, Berger J, Gilkeson GS, Keil DE. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. Toxicol Sci. 2008;104(1):144–54.
44. Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jorgensen E, et al. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. J Immunotoxicol. 2016;13(2):270–3.
45. Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, et al. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci. 2014;138(1):76–88.
46. Mogensen UB, Budtz-Jørgensen E, Heilmann C, Nielsen F, Weihe P, Grandjean P. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated compounds. Environ Health. 2015;14:47.
47. Grandjean P, Heilmann C. Perfluorinated compounds and immunotoxicity in children—reply. JAMA. 2012;307:1910–1.
48. Granum B, Haug LS, Namork E, Stolevik SB, Thomsen C, Aaberge IS, et al. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol. 2013;10(4):373–9.
49. Grandjean P, Heilmann C, Weihe P, Nielsen F, Mogensen UB, Timmermann A, et al. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J Immunotoxicol. 2017;14(1):188–95.
50. Mogensen UB, Grandjean P, Nielsen F, Weihe P, Budtz-Jorgensen E. Breastfeeding as an exposure pathway for perfluorinated alkylates. Environ Sci Technol. 2015;49(17):10466–73.
51. Dalsager L, Christensen N, Husby S, Kyhl H, Nielsen F, Host A, et al. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1–4 years among 359 children in the Odense child cohort. Environ Int. 2016;96:58–64.
52. Fei C, McLaughlin JK, Lipworth L, Olsen J. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res. 2010;110(8):773–7.
53. Bach CC, Henriksen TB, Bossi R, Bech BH, Fuglsang J, Olsen J, et al. Perfluoroalkyl acid concentrations in blood samples subjected to transportation and processing delay. PLoS One. 2015;10(9):e0137768.
54. Impinen A, Nygaard UC, Lodrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS, et al. Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ Res. 2018;160:518–23.
55. Goudarzi H, Miyashita C, Okada E, Kashino I, Chen CJ, Ito S, et al. Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4 years of age. Environ Int. 2017;104:132–8.
56. Hakim H, Provencher T, Chambers CT, Driedger SM, Dube E, Gavaruzzi T, et al. Interventions to help people understand community immunity: a systematic review. Vaccine. 2019;37(2):235–47.
57. Steenland K, Zhao L, Winquist A. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occup Environ Med. 2015;72(5):373–80.
58. Steenland K, Zhao L, Winquist A, Parks C. Ulcerative colitis and perfluorooctanoic acid (PFOA) in a highly exposed population of community residents and workers in the mid-Ohio valley. Environ Health Perspect. 2013;121(8):900–5.
59. Steenland K, Kugathasan S, Barr DB. PFOA and ulcerative colitis. Environ Res. 2018;165:317–21.
60. Fairley KJ, Purdy R, Kearns S, Anderson SE, Meade BJ. Exposure to the immunosuppressant, perfluorooctanoic acid, enhances the murine IgE and airway hyperreactivity response to ovalbumin. Toxicol Sci. 2007;97(2):375–83.
61. Timmermann CA, Budtz-Jorgensen E, Jensen TK, Osuna CE, Petersen MS, Steuerwald U, et al. Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination. J Immunotoxicol. 2017;14(1):39–49.

62. Pennings JL, Jennen DG, Nygaard UC, Namork E, Haug LS, van Loveren H, et al. Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. J Immunotoxicol. 2016;13(2):173–80.
63. National Toxicology Program. Immunotoxicity associated with exposure to perfluorooctanoic acid (PFOA) or perfluorooctane sulfonate (PFOS). Raleigh: National Toxicology Program; 2016. Contract No.: 17 May.
64. GBD Risk Factors Collaborators, Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(10010):2287–323.

Chapter 9
Long-Term Implications of Developmental Programming and Obesity

Jerrold J. Heindel

Abstract There is a global obesity pandemic. The prevailing theory is that obesity is the result of overeating and lack of exercise. However, the imbalance of calorie consumption and exercise per se cannot explain the reasons for the obesity epidemic. The susceptibility to obesity, like many noncommunicable diseases, has been shown to start during development due to poor nutrition, stress, or exposure to environmental chemicals. The subclass of environmental chemicals that can increase the susceptibility to obesity following exposure in utero or early life are called obesogens. There are now significant animal and human data indicating a role for obesogens in the obesity pandemic. This review presents an overview of the importance of exposure to obesogens in the etiology of obesity. The possibility of reducing exposures to obesogens during development could lead to a focus on prevention of obesity.

Keywords Environmental chemicals · Developmental programming · Obesogens · Obesity · Birth cohort

9.1 Introduction

Obesity is a global pandemic that affects humans of all ages. Indeed there are now more obese and overweight people than those that are underweight [1]. According to a recent report from the Global Burden of Disease Collaborators, in 2015, a total of 107.7 million children and 603.7 million adults were obese worldwide [2]. The prevalence of obesity globally in 2015 was 5% among children and 12% among adults. It has doubled in more than 70 countries since 1980 including many Asian countries. The highest rates of adult obesity occur in Egypt (35.5%), and the highest

J. J. Heindel (✉)
Healthy Environment and Endocrine Disruptor Strategies, Bolinas, CA, USA

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_9

percentage of childhood obesity occurs in the USA (12.7%) [2]. The USA and China have the highest number of obese adults, and China and India have the highest number of obese children. This excess body weight accounts for about 4 million deaths and 120 million disability-adjusted life years worldwide in 2015: about 75% of the deaths were due to cardiovascular disease [2]. Obesity is a risk factor for hypertension, dyslipidemia, insulin resistance, and non-alcoholic fatty liver diseases [3]. Obesity is also related to an increase in many cancers including colon, liver pancreas, breast, uterus, ovary, kidney, esophageal, gallbladder, and thyroid [2] as well as insulin resistance, hyperglycemia, and type 2 diabetes [4]. The cost of medical care attributable to obesity in the USA has been estimated at over USD 200 billion a year [5].

9.2 What Are the Causes of Obesity?

Obesity is a multifactorial disease with many interrelated causes [6, 7]. Fig. 9.1. There is certainly a genetic contribution to obesity [8, 9]. Twin studies indicate that from 40% to 70% of obesity may be due to genetics [10] however, it is essential to understand that twins have not only genetic similarities but also epigenetic similarities likely due to similarities in nutrition and stress and perhaps environmental chemical exposures. Also, the range of 40–70% leaves a significant portion of obesity to non-genetic causes. It is not clear how important genetics is to obesity since genome-wide association studies (GWAS) have identified over 300 single nucleotide polymorphisms in genes which can explain less than 5% of the heritability of

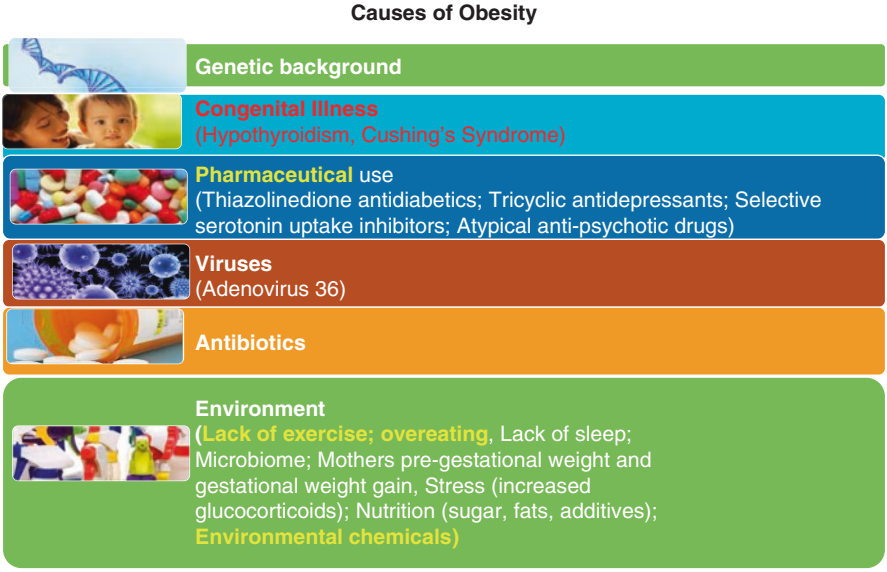


Fig. 9.1 This figure shows that obesity is multifactorial with many interacting factors

obesity [7]. The most prevalent genetic causes of obesity are related to mutations in the MC4R and FTO genes [7, 11]. It is also important to note that while genetics plays a key role in obesity, it cannot be the cause of the recent pandemic of obesity since genetics cannot change over such a short time (e.g., decades).

Many psychiatric drugs, including some mood stabilizers, antipsychotics, and antidepressants, can lead to weight gain and even obesity in some patients [12–14]. Also, some antihypertensive and antidiabetic (thiazolidinediones including rosiglitazone) drugs can lead to weight gain [15]. Corticosteroids which are used to treat inflammation will also cause significant weight gain likely mediated via appetite stimulation [16]. The fact that there are drugs designed for a specific purpose that have a side effect of causing weight gain is proof of principle for the ability of chemicals to have side effects that result in weight gain.

There is a causal relationship between obesity and adenovirus 36 [17, 18] in humans and animals. This virus has been shown to activate lipogenic and proinflammatory pathways in adipose tissue while at the same time improving insulin sensitivity, lipid profiles, and hepatic steatosis [19].

An altered microbiota favors an inflammatory status, influences energy extraction, and impairs metabolism of some nutrients and is therefore of substantial importance to the etiology of obesity [20]. Indeed, it is possible to transmit obesity via intact bacterial components of fecal microbiota from an obese animal to a lean animal [20]. There are strategies to use gut microbiota for the modulation of obesity in humans [21]. The intestinal microbiota acquired from the mother at birth and nurtured by breastfeeding play an essential role in metabolic programming. Antibiotic use in children alters the gut microbiota and thereby predisposes them to overweight [22]. There is a strong association between lifetime antibiotic use and weight gain when the child received the antibiotics early in life. The most significant effects happened when the first antibiotic exposure occurred in the first six months, and there were multiple episodes of antibiotic use in the first two years [23]. Indeed, antibiotic use results in a reduction in bifidobacterial, which protects against diet-induced obesity, which leads to adiposity, or metabolic disease in children [22].

The prevailing theory is that obesity is the result of overeating and lack of exercise [10]. However, obesity is complex and other factors are also playing important roles [6, 7]. The increase in obesity in children under two years of age also suggests that overeating and lack of exercise is not the complete answer to the obesity problem [24, 25]. The imbalance of calorie consumption and exercise per se cannot explain the reasons for the obesity epidemic. Indeed a 2015 study showed that for a given calorie intake or physical activity the predicted BMI was significantly higher in 2006 than 1998. These data indicate that it is harder to maintain weight today compared to 30 years ago. It is necessary to eat less and exercise more today to maintain weight [26]. These data, along with the lack of an explicit genetic component, support the idea that some physiological change has occurred over the past decades that increases the susceptibility to gain weight. The current interest in reducing obesity by focusing on nutrition, exercise, surgery, or drugs has had limited success as indicated by the increasing prevalence of obesity worldwide also shows that obesity is not just a problem of overeating and lack of exercise.The authors suggest that less sleep, more stress and prescription drugs could play an important role

in obesity, but there is also the possibility that exposure to environmental chemicals has altered development of the metabolic system such that it is more susceptible to weight gain when individuals eat more and exercise less, e.g., an altered set point.

This review will focus on the role that exposure to environmental chemicals can play in the current obesity epidemic. There have been several recent reviews that cover various aspects of this topic, they offer a more complete assessment of the role of environmental chemicals and obesity and other metabolic disorders [27–34].

9.3 When Does Obesity Start?

The idea that exposure to environmental chemicals could lead to obesity caught the attention of researchers with the onset of the new field of developmental origins of disease, now called developmental origins of health and disease (DOHaD). This new paradigm for the etiology of disease states that development consists of highly regulated, coordinated, and orchestrated processes which are controlled by hormones and growth factors and is highly sensitive to perturbations by exogenous stimuli [31, 35]. This sensitive time or window (e.g., in utero and early childhood when tissues are after developing) is sometimes called the first 1000 days [7]. Exposures to environmental stressors including environmental chemicals, nutrition, and stress during development can disrupt gene expression resulting in altered protein levels, cell numbers fate, or location to cause changes in cell and tissue function (in many cases without changes in phenotype). Many of these altered programming changes persist leading to increased sensitivity to disease and dysfunction across the lifespan [7, 35, 36]. Environmental chemicals can alter developmental programing of adipose tissue as shown by recent data on tributyltin. Tributyltin exposure to mesenchymal stem cells or 3T3L1 cells leads to their differentiation into adipocytes that store fat but are functionally deficient with abnormal metabolism [37–40]. This is a great example of an environmental chemical interfering with programming resulting in functional changes in adipose tissue that can increase susceptibility to obesity later in life. Indeed through 2014, 425 epidemiologic prospective cohort studies examined the association of exposure to environmental chemicals and a variety of health outcomes [41] including neurological/neurobehavioral, reproductive, immune, cancer, endocrine, cardiovascular, respiratory, and obesity. These epidemiology studies have studied over 60 different environmental chemicals for their association with diseases. Epigenetic changes appear to underlie the changes in gene expression due to environmental stressors, and many of the functional changes that result are sexually dimorphic and are latent, not becoming apparent until later in life [42]. However, it is not clear which epigenetic marks are the most important in the programming of obesity. Likely all the epigenetic marks, DNA methylation, histone modifications, and non-coding RNAs will be shown to be involved [43]. The determination of the role of epigenetics in obesity programming will require animal studies where changes in epigenetic marks in specific genes in adipose tissue and other metabolically essential tissues can be directly measured and related to disease

outcomes. The assessment of epigenetic changes in blood of humans and relating those changes to the obesity phenotype has not been validated to reflect what is happening in the tissues of concern.

There are likely other sensitive windows for the development of obesity including preconception [44] and preadolescence [7]. Recent reviews cover the history and importance of the DOHaD paradigm in the etiology of a wide variety of noncommunicable diseases [45–47]. A proof of principle for the importance of development in the etiology of diseases comes from the animal and human data on diethylstilbestrol (DES). DES is an estrogenic drug given during pregnancy that resulted in a rare vaginal cancer and reproductive abnormalities in the sons and daughters and animal models [48, 49]. It is now clear from human and animal studies that obesity is one of the diseases that has its origins during development [31].

9.3.1 The Obesogen Hypothesis

Only a decade ago data from the Blumberg laboratory [50] led them to propose a new theory for obesity. They called it the obesogen hypothesis, and it comes directly from the DOHaD paradigm. This hypothesis states that there is a subset of environmental chemicals, obesogens, which can act during development to disrupt adipose tissue development, leading to an increase in fat cells. Obesogens can also alter food intake and metabolism via effects on the development and function of the pancreas, liver, gastrointestinal tract, muscle, and brain thereby altering the programming of the "set point" sensitivity for developing obesity later in life. This new hypothesis does not dismiss the importance of food intake and exercise in the etiology of obesity but states that environmental chemicals can influence how much food intake it takes to put on weight and how much exercise is needed to reduce weight via alterations in developmental programming that are permanent. Fig. 9.2.

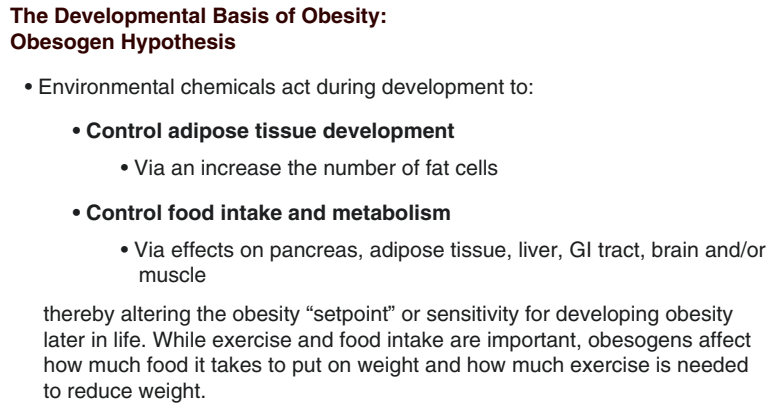


Fig. 9.2 This figure shows the components of the Obesogen Hypothesis for obesity. This hypothesis posits that environmental chemicals can affect all aspects of metabolism resulting in altered sensitivity for developing obesity

The two critical components of the obesogen hypothesis are that obesity starts during development and that the altered developmental programming is due to exposure to environmental chemicals that interfere with the hormonal control of the various aspects of metabolism. This review overviews the data supporting the obesogen hypothesis.

9.3.2 Linking Environmental Chemicals to Obesity

Data supporting a role for chemicals in weight gain first appeared in the literature in the 1980s and 1990s toxicity studies. As noted in Baillie-Hamilton [51] toxicity studies in adult rodents showed that some pesticides including organophosphates and carbamates, plastics, heavy metals, solvents, and polybrominated biphenyls and polychlorinated biphenyls at high doses resulted in weight gain instead of the expected weight loss. However, these data were not considered important and were not followed up as the focus of these toxicity studies was on reducing weight and not on gaining weight.

Nonetheless, these data indicated that some doses of a variety of environmental chemicals could cause weight gain in adults. The first animal studies that showed an environmental chemical could cause obesity because of exposure during development were the result of serendipity [52, 53]. Each of these laboratories was studying the effects of developmental exposure to bisphenol A on some aspect of reproduction when they noticed the animals also gained weight.

There is now significant evidence showing an association between exposure to a variety of environmental chemicals during development with obesity in both animal models and human epidemiology studies as noted in the following reviews [28, 30–32, 34, 54–56].

9.3.3 Smoking

The most robust data supporting a role for environmental chemicals causing obesity comes from studies of smoking and nicotine. Maternal smoking during pregnancy increases the risk of obesity in children in over 20 epidemiology birth cohort studies [57, 58]. This effect of smoking during pregnancy to cause weight gain in children occurs even if smoking is limited to early pregnancy [59]. Also, nicotine exposure during development in animal models also results in weight gain in the male offspring [60, 61] ; the mechanism appears to be via an alteration in early adipogenesis with no effect on food intake [61]. These studies also showed that prenatal nicotine exposure decreased spontaneous physical activity later in life and resulted in animals that required less high fat food intake to gain weight [60]. These data highly suggest that the active ingredient in tobacco smoke causing weight gain is nicotine. It is noteworthy that, as with many environmental chemicals, the effects of smoking

during pregnancy on the offspring are different from the impact of smoking in adults, where it seems to reduce weight gain.

9.4 Persistent and Bio Accumulative Environmental Chemicals

Persistent organic pollutants (POPs) are a class of chemicals which are persistent in the environment and which have an extremely long half-life in humans and animals. There have been many POPs banned because of their persistence and bioaccumulation. POPs are taken up by adipose tissue and stored in lipid droplets where they can cause toxicity to adipocytes and be released back into the circulation on weight loss to cause toxicity [62, 63]. While most studies of POPs have focused on specific compounds within this classification, there are data related to POPs exposure as a mixture [64]. Exposure of adult rats to a high fat diet containing mixtures of POPs found in farm-raised salmon for 28 days(via feeding of crude or refined oil from contaminated salmon) led to insulin resistance, abdominal obesity, and hepatosteatosis [65, 66]. Adult rats fed actual salmon fillets containing high concentrations of POPs [66] caused similar effects. These studies support not only that POPs can cause metabolic abnormalities but also that rats are sensitive to their metabolic effects in adulthood and that the POPs overcame the protective effects of unsaturated fatty acids in the salmon. Further Hong et al. [67] showed that POPs may counter the positive effects of weight loss on hepatic and serum lipids. In general, POPs whether stored in adipose tissue or when in the blood cause toxic effects which can lead to altered metabolism and weight gain. In a related phenomenon, release of POPS into the circulation during weight loss can lead to cycles of weight loss and gain [68, 69]. An intriguing explanation for this important health problem of weight loss and gain may be related to POPs: POPs released from adipose tissue during weight loss if not eliminated from the body could stimulate weight gain. As reviewed by Lee et al. [68] this problem is likely more pronounced in the elderly as they are likely to be more contaminated with POPs and they have reduced ability to metabolize and excrete the POPs released into the circulation.

9.4.1 DDT/DDE

DDT is a potent insecticide POP which was banned in the USA in 1972 partially due to its effects on bird egg shell thinning which raised concerns about its impact on animal and human health reviewed in [70]. Because of its long half-life, DDT is still present in human populations today [71]. Several prospective cohort studies have reported that developmental exposure to DDE, the major metabolite of DDT, is associated with weight gain in infants, and overweight in children that persists into adulthood and is associated with measures of abdominal fatness [72–77]. These

data are especially troubling since DDT use has been banned in most countries for several decades. Some studies found sex differences with effects only in females [72, 74, 75]. Animal data also supports a role for DDT metabolites in the obesity epidemic. Perinatal exposure to DDT in mice increased adiposity in young female mice and led to reduced core temperature and energy expenditure due to impaired thermogenesis leading to a risk for metabolic syndrome [78]. DDE stimulated lipid accumulation in 3T3L1 preadipocytes [79]. DDT has been shown to induce adipocyte differentiation via increased PPAR g and C/EBPa expression [80]. The effects of developmental exposure to DDT have also been shown to be transmitted transgenerationally to future generations [81]. Thus, data support DDT(DDE) as an animal and human obesogen.

9.4.2 PFOA/PFOS

PFASs (PFOAs and PFOS) are POPs used as stain resistant coatings on paper, furniture, and clothing resulting in human exposures from contaminated food, water, dust, and soil [82–84]. There are systematic reviews of the effects of PFOA on fetal growth [83, 85] which indicate that there is a high level of confidence that PFOA causes decreased fetal growth in both human and non-human species. Low birth weight has been shown to lead to catch up growth and increased weight gain in humans. Effects on long-term weight could be determined since these studies have not been followed up across the lifespan.

Also, a recent meta-analysis of ten prospective cohort studies showed that early life exposure to PFOA is associated with an increased risk for childhood obesity (ages five to nine) [86]. One study [87] showed that developmental exposure to PFOA lead to increased BMI and waist circumference in females only at 20 years old. All the epidemiology studies noted an increased sensitivity of females to PFAS exposures. One animal model study also showed that chemicals in this class could be obesogens, with a nonmonotonic dose response on weight gain in CD-1 mice in mid-life [88]. Perfluorobutanesulfonic acid (PFBS) used as a replacement for PFOS promoted the differentiation of 3T3L1 preadipocytes to adipocytes [89] and perturbed energy homeostasis in zebrafish [90]. Also, PFOA may activate PPAR g, a master regulator of fat cell differentiation from stem cells [91]. Overall it is likely that PFOA and other PFASs are weak obesogens.

9.4.3 PCBs

PCBs are a large class of lipophilic POPs used in electrical equipment that were banned over 35 years ago because of their bioaccumulation and long half-life in humans. Nonetheless, they remain in the environment and people today because of contamination of the food chain including fish, meat, and milk [64]. They are stored

in adipose tissue depending on their individual toxicokinetic properties [68] and are continually release into the blood especially during weight loss [63]. Specific PCBs stored in adipose tissue cause alterations in lipid droplet formation and fatty acid mobilization, impairment of triglyceride synthesis, and enhancement of adipocyte differentiation, all of which could lead to altered regulation of energy homeostasis and contribute to obesity [62, 78]. Some PCBs have dioxin-like properties and like dioxin have been shown to be obesogens under certain conditions reviewed in [63]. PCB-77 induces adipocyte differentiation and proinflammatory cytokines in vitro and promoted weight gain in adult wild type mice, an effect that was abolished by the AhR antagonist alpha-naphthaflavone, but not in AhR negative mice. These data indicate that the effects of this particular PCB are due to its dioxin-like activity [92]. Overall PCBS are obesogenic. However, the mechanism is not defined.

9.4.4 PAHs (Air Pollution)

Air pollution is a complex mixture of organic chemicals, polycyclic aromatic hydrocarbons (PAHs), and particulate matter (PM), with the most common PM being PM 2.5 Air pollution is a huge global problem data affecting billions of people. Therefore, weight gain due to exposure to air pollution must be taken seriously. Adult exposure [93, 94] or early life exposure to air pollution (PM 2.5) can cause weight gain in rodent offspring [94]. Prenatal air pollution (diesel exhaust particles) resulted in weight gain in adult male mice [95, 96]. In utero exposure to benzo(p) pyrene (a polycyclic aromatic hydrocarbon, PAH found as byproducts of burning of biomass or fossil fuels including diesel fuel), increases adiposity and hepatic liver accumulation in adult female mice [97, 98]. Using a rat model, Wei et al. [99] showed that prenatal exposure to Beijing's polluted air resulted in increased weight in the offspring at eight wks. of age. Thus, there are animal data supporting air pollution or components of air pollution as obesogens.

There are also a significant number of birth cohort studies supporting air pollution, and various components of air pollution are obesogens. Prenatal and early life exposure to air pollution, (PM 2.5) or [100] PAHs during pregnancy, assessed by personal monitoring, is associated with obesity at ages 5 and seven years. As noted by McConnell et al. [101] the findings that components of air pollution have obesogenic effects in humans could have large public health implications.

9.5 Nonpersistent and Non-bio Accumulative Environmental Chemicals

Chemicals that are non-persistent and non-bio accumulative are more difficult to measure accurately in human prospective cohort studies than persistent chemicals. Thus, it is critical to have multiple measurements across the exposure period to

ensure adequate statistical power for the chemical measurements. These short lived chemicals like BPA, phthalates, triclosan, parabens, PM 2.5, etc. may require at least ten exposure estimates per individual [102]. Since many epidemiology studies do not have the needed number of estimates of exposure the results of these studies are suspect; improved exposure assessments are needed for future studies.

9.5.1 BPA

Bisphenol A (BPA)is ubiquitously found in the environment. It can be found in human blood, urine, and umbilical cord, placenta and breast milk [103, 104]. In the USA BPA has been detected in over 95% of people examined by the CDC biomonitoring studies [105]. Human exposures come from polycarbonate plastic, expoxy resins and cash register receipts [103, 106] resulting in exposure via food, water, and skin. BPA has estrogenic activity [103] but is not a classical estrogen. It also can activate glucocorticoid receptors, peroxisome proliferator-activated receptor gamma (PPAR g), thyroid receptors, and retinoid receptors [34, 107, 108]. It is notable that all these receptors are known to play a role in adipogenesis. BPA increases gene expression, and enzyme activity of a variety of genes involved in cell differentiation and fat storage induces adipocyte differentiation and increases the release of proinflammatory cytokines in several in vitro cell systems including 3T3L1 cells [109] and human adipose stem cells/primary human preadipocytes [110, 111]. It is notable that the glucuronide of BPA which is thought to be an inactive metabolite can also increase adipogenesis in 3T3L1 cells [112, 113] as well as the BPA replacement BPS [113]. Numerous developmental studies in rodent models have shown that BPA can be an obesogen [107, 114–116]. Not all studies have shown an effect of BPA on weight gain, and that is to be expected because of the differences in modes of exposure, timing of exposure BPA, doses and dose ranges, some studies have found a nonmonotonic dose response [114, 116], animal models of differing sensitivity endpoint measured (weight or fat), timing of measurement and diet (reviewed in [107]). A few studies have provided data on potential mechanisms of BPA about causing weight gain. Two studies stand out because of their direct relevance to the human situation. Desai et al. [117] showed that developmental exposure to BPA in a rat model increased food consumption which resulted from effects of BPA in the brain to cause an increase in the number of appetite neurons and a decrease in the number of satiety neurons. Johnson et al. [118] showed that developmental exposure to BPA in their mouse model resulted in females that spent more time sleeping, walked slower, traveled less and overall used less energy, similar to what has been called a “couch potato” in humans. Thus, the preponderance of data from in vitro and in vivo animal studies indicate that BPA is an obesogen, with sex specific effects that are dose dependent and likely involves integration of effects on several tissues via a variety of mechanisms.

The human studies of BPA exposure in prospective cohort studies (reviewed in 2017) [56] have been inconsistent with some studies showing increased body fat at age 7 [119] or BMI [120] while others did not find any positive effects on body weight or body fat [56, 121].

Since BPA has a short half-life of about 2 h, does not bioaccumulate and there are considerable daily variations in exposures, it is difficult to get a measurement of exposure that reflects exposure throughout pregnancy or even a specific trimester. Further, the measurement of weight gain is insensitive, and thus more sensitive endpoints must be measured including changes in gene expression. Until there are studies with improved exposure assessment and more sensitive endpoints, it will not be possible to determine if indeed BPA is a human obesogen.

9.5.2 Phthalates

Phthalates are a class of chemicals that are used as plasticizers, found in many consumer products including toys, plastic tubing of various kinds, vinyl flooring, as well as adhesives, detergents [122]. Medications, and personal care products [122] also contain phthalates. The higher molecular weight phthalates are used as plasticizers, while the lower molecular weight phthalates are used in personal care products including as a carrier in cosmetics. According to NHANES [123] virtually all individuals examined have a variety of phthalates in their bodies. Mixtures of phthalates to which people are usually exposed are rapidly hydrolyzed into their monoesters. It is the monoesters that are measured in urine to assess exposure to phthalates and multiple phthalates monoesters must be measured to determine overall phthalate exposure and then exposure to specific phthalates. Since phthalates have a short half-life of less than 24 h, it is difficult to get an accurate measure of exposure without multiple measurements throughout pregnancy.

Prospective cohort studies assess exposure to multiple phthalate monoesters in urine and use that information to determine which phthalates are associated with a health outcome. Of all the phthalates assessed, prenatal exposure to diethylhexlphthalate (DEHP) has been associated with weight gain, obesity, and central fatness in several large epidemiology studies in various countries. Developmental exposure to DEHP or its metabolite MEHP, have also been shown to result in weight gain, an increase in fat depots and an increase in numbers and size of adipocytes in males of several strains of mice [124–126]. In utero exposure to 50ug/kg/day, DEHP (a very low dose) led to an increase in visceral fat and leptin levels in male and female C57BL/6J mice [127]. Mechanistically, MEHP has been shown to increase the expression of PPAR g and increase the differentiation of 3T3L1 preadipocytes and multipotent mesenchymal stem cells into adipocytes individually [128–131], and in combination with BPA and tributyl tin [130]. These data provide at least one possible mechanism for the increased weight gain in the human and animal studies.

Other mechanisms supported by in vitro, animal and human studies include, antiandrogenicity [132] thyroid hormone [133, 134], activation of estrogen receptors [135] induction of reactive oxygen species [136, 137] alteration of glucose homeostasis [138] and effects on feeding behavior [34]. DEHP can also increase body weight and adipose tissue because of adult exposure to C3H/He mice [139]. A systematic review and meta-analysis of early life exposure to DEHP showed that early life exposures were significantly associated with increased fat weight across 31 studies [140]. Thus human, animal data and in vitro data provide strong evidence that exposures to specific phthalates can lead to obesity later in life. Thus at least some phthalates are obesogens. Nonetheless, there is a need for more studies. Specifically, more prospective birth cohort studies with improved exposure assessments that measure multiple overlapping endpoints related to weight gain, glucose tolerance and liver function over a longer time frame than childhood are needed.

9.5.3 Tributyl Tin

Tributyl tin (TBT) is environmental chemical characterized as an endocrine disruptor because of its actions as an agonist with the RXR/PPARg receptor which plays an important role in directing stem cells towards becoming adipocytes [141]. It is a used as a fungicide and is found along with other tin compounds in vinyl [141]. It has been shown to increase the differentiation of adipocytes from precursor cell lines or mesenchymal stem cells [142, 143] and in vivo to alter developmental programming of adipose tissue leading to increased weight of fat tissue in mice [50, 144] zebrafish [145]. The Blumberg lab in 2006 called TBT an obesogen which started the focus on chemicals that can cause obesity [50]. Indeed, TBT has the strongest in vitro and animal data from multiple studies and multiple labs showing that it is an obesogen [37, 141, 146]. There are now data showing that the obesogenic effect of TBT can extend over multiple generations indicating that it is a transgenerational obesogen [147]. Transgenerational effects may be transferred via the male or female gametes. However, there are essentially no human data due partly to the lack of a sensitive and available assay for TBT in biological fluids including blood and urine. Therefore, it is not known whether TBT is a human obesogen.

9.5.4 Parabens

Parabens are widely used as preservatives in foods, drugs, and personal care products reviewed in [148] and have been shown to have estrogenic and antiandrogenic properties, and can activate PPAR g and the glucocorticoid receptor indicating they might be obesogens. Serum, urine, and breast milk may contain

parabens [149, 150]. They have been shown to promote adipocyte differentiation in vitro in 3T3L1 cells, primary cultures from human adipose tissue, and the multipotent stem cell line, C3H10T1/2 [148, 151, 152] likely through glucocorticoid receptor or PPAR g. Like TBT, parabens stimulate multipotent stem cells to differentiate into adipocytes at the expense of osteoblasts or chondrocytes [152]. There are no animal studies on parabens and obesity. In a prospective birth cohort study, methylparaben levels were associated with birth length but not weight [153]. In another study parabens were associated with weight at birth and this association remained through 3 years. of age [154] but the subjects were not followed up later in life to see if the weight gain persisted. While the in vitro data suggest parabens should be obesogens, there is a lack of human and animal studies to show if these effects would translate to the in vivo situation.

9.5.5 Triclosan

Triclosan is a synthetic broad-spectrum antimicrobial agent that is added to thousands of personal care products and impregnated into some clothing and food packaging [155]. Triclosan is an endocrine disruptor with estrogenic and thyroid activity and carcinogen in rodents [155, 156]. Human blood, urine, and breast milk have been shown to contain triclosan [155, 157]. Indeed in one survey in the US 74.6% of the population had detectable levels in their urine [158]. It has been shown to be a reproductive and developmental toxicant [156] acting at least partially via alterations in serum thyroxine levels. As noted in a recent review by Braun [56], there is insufficient evidence to associate developmental exposure to triclosan with childhood obesity. There are only two prospective cohort studies, and they were negative [154, 159]. However, they both used one spot urine sample as the measure of triclosan during pregnancy; which is not optimal for a non-persistent chemical with a short half-life. Thus, more data with improved exposure assessments are needed. There are no in vitro or animal studies that have examined the possibility of triclosan to affect weight gain which is concerning considering the extent of human exposure.

Other chemicals implicated in stimulating weight gain in animal models or in vitro systems include nonylphenol [160], the herbicide atrazine [161], the fungicide triflumizole [162], pesticide permethrin [163] the insecticide deltamethrin [164],insecticide fipronil [165], lead [166], monosodium glutamate [167], high fructose corn syrup [168], flame retardants [169–171], badge [172], endrin [173],imidacloprid neonicotinoid insecticide [174], artificial sweeteners [175–177], BPS (replacement for BPA) [178], BPA glucuronide (“inactive metabolite” of BPA) [112], and house dust [179]. Many of the effects of obesogens, as expected, are sex specific with effects in either males or females [31]. Fig. 9.3 provides an overview of obesogens.

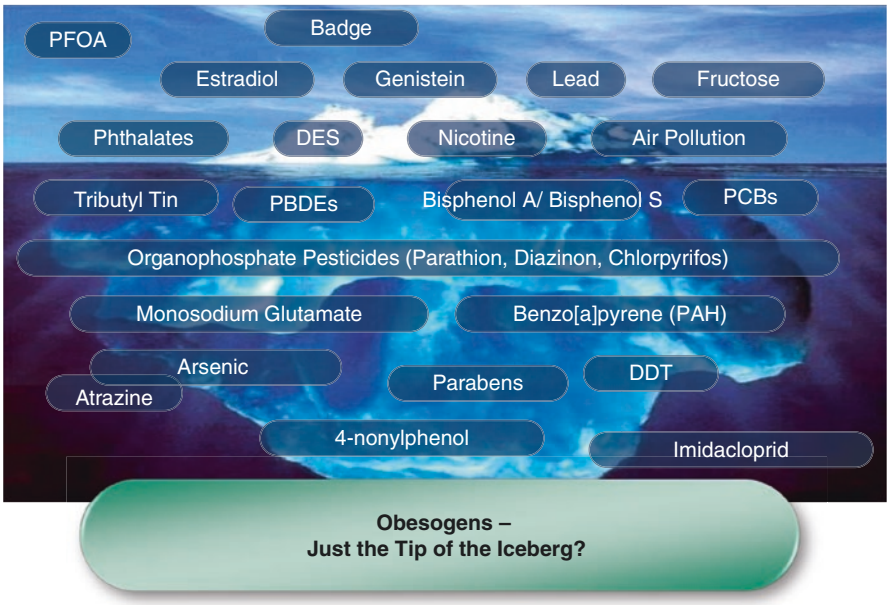


Fig. 9.3 There are currently about 50 chemicals and categories of chemicals that have been identified as obesogens. Every year more chemicals are identified as obesogens. We propose that what we currently know is only the tip of the iceberg. That is, it is likely there will be many more chemicals that are obesogens, the more we look the more we will find

9.6 Transgenerational Obesity

The most exciting and at the same time disturbing data relating to obesogens is that their effects can be transferred to future generations in animal studies: a phenomenon called transgenerational inheritance [180, 181]. Transgenerational inheritance means that an exposure of a pregnant dam to an obesogen not only can cause obesity in the offspring but also affects future generations such that the effects can occur in grand-offspring or second generation and great grand- offspring or third generation [182]. This transgenerational inheritance of obesity has now been shown for TBT [147], DDT [81], a combination of BPA and two phthalates DEHP and DBP [183] and a hydrocarbon mixture(jet fuel) [184] but not with dioxin, vinclozolin, or a permethrin/DEET mixture indicating some specificity to the effects on weight gain [185]. A major question is what chemicals can cause transgenerational inheritance of obesity? Transgenerational effects are likely to be at least partially epigenetic in origin with both chromatin remodeling and DNA methylation changes playing a role, although the specific epigenetic marks responsible have not been defined.

9.7 Relation to the Human Situation

In humans it is easy to gain weight, hard to lose weight and even harder to maintain weight loss. As we all know, it is easy to gain weight and difficult if not impossible to lose weight and to keep it off. A person who is obese has an annual chance of losing 5% of total weight of only 15% [186].

While the reasons for weight related health problem are multifactorial, as noted above, it is becoming clear that developmental programming of metabolism Is likely responsible. Developmental exposure to environmental chemicals, as well as abnormal nutrition or increased stress, can result in altered programming of adipocytes leading to increased adipose tissue, also altered pancreas, liver, GI tract and brain functions that lead to altered set points for gaining and losing weight. Indeed, many of the differences noted between obese and normal weight individuals can be the result of altered programming of metabolism. Some of the differences are psychological while others are biochemical. Obese individuals prefer high fat and high sugar foods; they have a higher desire and anticipation for food, will work harder for food, get a greater reward from food intake and tend to be emotional eaters with increased eating in response to anxiety, depression, and mood swing [187, 188]. On the biochemical side, obese individuals have higher insulin levels and insulin resistance, [189], Impaired leptin signaling [190], decreased striatal dopamine (DR2) receptors and increased dopamine receptors in brain and pleasure centers [191]. It is important to show whether these changes are due to altered programming of metabolism and whether environmental chemicals play a role in the altered programming that leads to increased susceptibility to weight gain and reduced sensitivity to weight loss. Body weight set point is modifiable by environmental factors and that environmental factors, including obesogens, can lead to a metabolic shift in set point that the body will defend [192] which support the obesogen hypothesis.

As noted above obesogens can cause weight gain leading to obesity when the exposure is in utero or early childhood in animal and human studies. However, in the human situation increased weight gain may not occur until later in life. For example, developmental exposure to diethylstilbestrol, a strong estrogenic drug, causes weight gain but not until the animal gets a second it, and that second hit is the increased estrogen in females at puberty. Developmental exposure to DES alters programing of the estrogenic pathways thereby sensitizing the animal to estrogen so at puberty fat cells that have estrogen receptors respond with increased fat uptake resulting in a mouse that at 9 months of age can weigh close to 90 grams compared to controls at around 30 gms [193] a situation that matches what happens in humans.

Another effect that occurs in animals due to an obesogenic chemical that matches the human situation is altered programming that leads to increased food intake. Indeed, developmental exposure of a rat model to BPA leads to weight gain later in like due to increased food intake. BPA-induced changes in the appetite and satiety neurons in the brain can lead to increased food intake. BPA exposure leads to a reduction in the number of satiety neurons and an increase in the appetite neurons [117]. In an in vitro model of adipogenesis, BPA increased adipocyte differentiation, but the resulting adipocytes dysfunctional, with impaired insulin signaling and increased inflammatory cytokine gene expression [40]. Similarly, 12 weeks of expo-

sure to p, pDDE in Wistar rats led to alterations in the gene expression and proinflammatory cytokines in adipose tissue indicating that the chemical cause metabolically dysfunctional adipocytes [194].

Reduced exercise has been promoted as a cause of obesity in humans. Again, we have animal data that developmental exposure to either BPA or nicotine can cause reduced activity leading to reduced energy expenditure throughout lifetime causing a "couch syndrome" in animals [60, 118].

There are also data showing that the weight gain resulting from developmental exposure to an obesogen is augmented by a high fat diet mimicking the human situation. Imidacloprid promotes high fat diet-induced obesity in mice [195]. Less high fat food is needed to cause weight gain in a mouse model of prenatal nicotine exposure [60]. Chammoro-Garcia et al. [196] showed that fourth-generation animals (TBT given to the pregnant dam that gave birth to the first generation animals) when given a diet with increased fat (from 13.2% fat to 21.6% fat) had an increased weight gain compared to the controls. These results indicate that the TBT exposure generations previously had set these animals up to gain weight easier. When the fat content of the diet of these animals was reduced back to 13.2%, the animals exposed to TBT four generations earlier lost the weight gained from the higher fat diet slowly indicating, like the human situation, that it was easier to gain weight and harder to lose weight. This lab also took the fourth-generation animals and fasted them overnight, and the control animals lost weight while the TBT exposed animals did not; mimicking the human situation from obesogen developmental programing of metabolism. These data are exactly what is needed to get the public attention to the importance of EDCs and obesogens, they provide real world examples of how exposure to obesogens can increase susceptibility to gain weight that everyone can relate too.

9.8 Metabolism Disruptors

While the focus of this review is on chemicals that cause obesity (obesogens), it is now clear that many environmental chemicals have multiple sites and mechanisms of action, just like the hormones they mimic or antagonize. Thus, a new classification of environmental chemicals, metabolism disruptors has been proposed. Metabolism disruptors are environmental chemicals that act on multiple tissues resulting in multiple diseases like obesity and diabetes, fatty liver and indeed metabolic syndrome [31]. As noted in a recent review many endocrine disruptors are not only obesogens but are more general metabolism disruptors that can lead not only to obesity but also type 2 diabetes, fatty livers, and cardiovascular effects. The endocrine disruptors with the strongest data indicating that they are general metabolism disruptors include bisphenol A, DEHP, DDT, TBT, air pollution, arsenic smoking/nicotine and PCBs [31, 197, 198]. The two chemicals with the strongest data supporting a role in both obesity and type 2 diabetes are BPA and PCBs. Imidacloprid, a neonicotinoid insecticide promotes high fat-induced weight gain and impaired glucose metabolism after 12 week adult exposure [195] and promotes adipogenesis in 3T3L1 cells [199]. Tolyfluanid, a

fungicide, promotes the development of metabolic disease from adult exposures [200]. These data indicate the importance of measuring both developmental and adult exposures along with multiple endpoints in toxicity studies as it is likely that chemicals with obesogenic activity will affect multiple receptor systems and thus multiple tissues and metabolic diseases. It is not clear whether the effects of these chemicals on tissues leading to obesity and type 2 diabetes are direct or indirect and whether the effects are additive or synergistic.

9.9 Obesogens and Receptors

As noted in a recent review [28] environmental chemical exposures during development can lead to increased weight gain via a variety of receptor-mediated pathways. Some obesogens increase the number and activity of adipose cells and alter other metabolic systems via activation of PPAR gamma (tributyl tin, parabens, phthalates), aryl hydrocarbon receptor (AhR) (dioxin-like chemicals including some POPs), and estrogen receptors (DES, bisphenol A, and phytoestrogens). Tolyfluanid, a fungicide, promotes adipocyte differentiation and increases weight gain in mice after adult exposures via the glucocorticoid receptor [200]. It is likely that as more chemicals are examined for their effects on obesity and metabolism more receptor systems will be shown to be involved. Indeed differentiation of 3T3 L1 preadipocytes requires activation of many receptors and pathways including GR, PPARg, RXRa, cAMP, insulin signaling and JAK-STAT as well as other pathways indicating that there are other potential pathways whereby obesogens could be acting [200] including FXR and thyroid receptor beta. An important point to note about environmental chemicals and receptor systems is that, unlike hormones, environmental chemicals can act through multiple signaling pathways and targets, reviewed in [34]. BPA, for example, while it is an estrogen, also affects androgen, thyroid, PPARg, LXR, PXR, progesterone, and Thy1 receptor pathways [32, 171]. EDCs that are obesogens can also act via oxidative stress and mitochondrial dysfunction [34, 201] and inflammatory pathways [202]. There is an urgent need to understand the various mechanistic pathways underlying how exposure to obesogens can predispose to obesity.

9.10 Perfect Storm for Obesity and Metabolic Diseases

It has been proposed that exposure to environmental chemicals (along with nutrition and stress) leads to a perfect storm for obesity [203] Fig. 9.4. During development obesogens probably along with poor nutrition and stress can result in altered programming of adipose tissue, pancreas, liver, GI tract and brain centers controlling appetite and satiety as well as reward pathways that control emotional eating and "addictive eating" resulting in an abnormal metabolic system with increased sensitivity to weight gain. Then throughout life increased consumption of high fat and sugar diets, reduced exercise and additional bouts of stress provides a "second hit"

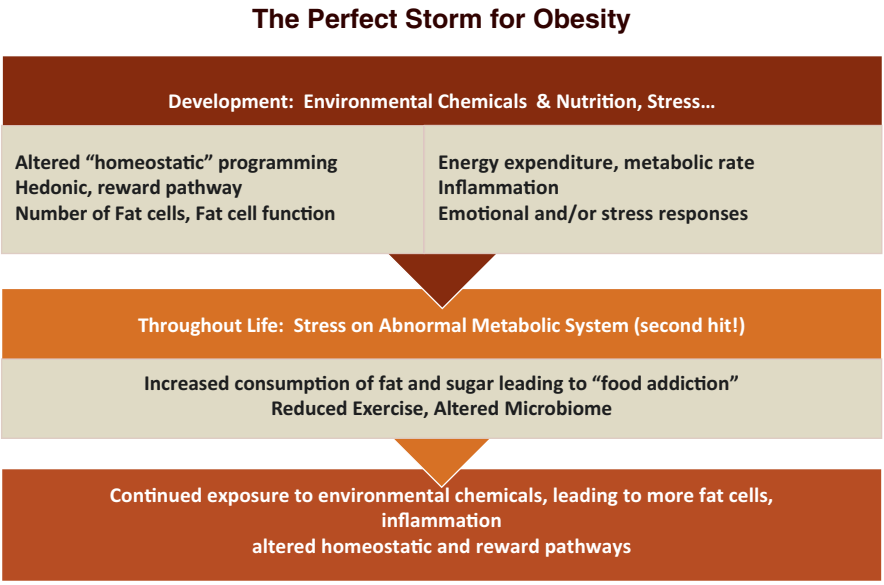


Fig. 9.4 Obesity is the result of many interacting factors across the lifespan. During development environmental chemicals and poor nutrition lead to altered epigenetic changes in the brain, adipose tissue and other tissues that lead to increased susceptibility or sensitivity to develop obesity later in life. Then throughout life poor diet, reduced excercise and altered microbiome interact with the increased sensitivity set up during development to result in increased sensitivity to develop obesity. Exposure to environmental chemicals throughout life also interacts with metabolism to again increase the susceptibility to obesity. The end result is an epidemic of obesity

on an already sensitized system leading to further weight gain and altered metabolism affecting many tissues including the liver, pancreas, and brain. Also, throughout life, there will be additional exposure to obesogens and more stress leading to more fat cells, altered glucose and fat metabolism leading to more susceptibility to weight gain and altered metabolism. Thus, obesogen exposures during development and throughout life lead to a metabolic system that has an increased sensitivity or susceptibility (perhaps set point) to weight gain, how much food intake is needed to lead to gain weight how much exercise is needed to lead to weight loss.

The situation is even worse as there is the possibility that obesogens can increase obesity not only over a lifetime but also across generations, as noted above. If that is indeed the situation, then we must ask whether life as we know it is sustainable with diseases increasing over generations. During each generation obesogen exposure during pregnancy increases susceptibility to obesity and the effects are transmitted across generations. The result will be that obesity risk will be compounded over generations leading to an unsustainable situation for human health. While at this time this paradigm is hypothetical, as there are no data specifically asking this question for obesogens and obesity, the possibility must be considered, and steps take to be sure it does not happen.

9.11 Windows of Opportunity

All the news is not bad as a window of sensitivity for disease is also a window of opportunity to prevent disease. Obesity, like many chronic noncommunicable diseases, has it origin during development, and there are significant data in animals supporting a role for obesogens to cause obesity. Thus, it is appropriate to discuss how we can use the information at hand to prevent obesity and metabolic diseases by focus on reducing exposures that can alter programming during pre-pregnancy, pregnancy, and the first years of life. In addition to obesogen exposures which is the focus of this review, nutrition can also alter developmental programming leading to increases susceptibility to obesity [204]. Since prevention is much more cost effective than interventions after the fact, a focus at the times of highest susceptibility is likely to have the biggest effect to prevent obesity. Thus, it is important to focus on maternal and paternal weight, and nutrition before pregnancy, overweight during pregnancy is another important risk factor for obesity in children as well as reducing stress and obesogen exposure during this same time frame. Proper nutrition and reduction of obesogen exposure is key. Some simple changes like using filtered water, eliminating plastic food containers, not microwaving in plastic, using organic cleaners and pesticides and using cosmetics and sunscreens that have reduced chemical contaminants can reduce obesogen exposures. During infancy and the toddler years, it is important to breast feed, to reduce when possible the use of antibiotics and to reduce obesogen exposure by reducing dust, vinyl products, carpets, and toxic chemicals in toys like lead, and phthalates from some plastic products. Then throughout life to continue to be mindful of toxic chemical exposures in the air, water, and food around the house, in the yard and to watch nutrition (reduce high fat diets and high sugar foods) that can provide a second hit to a system already sensitive to weight gain.

9.12 Conclusions

The obesogen field is only a little over ten years old. There have been many significant discoveries during this first decade of research including identification of obesogens in animal and human studies, and a focus on development as the sensitive period for obesogens to act. Developmental exposure to a wide variety of environmental chemicals can cause or increase the susceptibility to weight gain in animals and humans. As the obesogen field moves into its second decade, the real question is to what extent do obesogens contribute to the obesity epidemic [205].

Conflict of Interest The author declares no conflict of interest.

References

1. Ogden CL, Carroll MD, Fryar CD, Flegal KM. Prevalence of obesity among adults and youth: United States, 2011–2014. NCHS Data Brief. 2015;219:1–8.
2. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Lee A, et al. Health effects of overweight and obesity in 195 countries over 25 years. N Engl J Med. 2017;377(1):13–27.
3. Almeda-Valdes P, Aguilar-Salinas CA, Uribe M, Canizales-Quinteros S, Méndez-Sánchez N. Impact of anthropometric cut-off values in determining the prevalence of metabolic alterations. Eur J Clin Investig. 2016;46(11):940–6.
4. Legler J, et al. The OBELIX project: early life exposure to endocrine disruptors and obesity. Am J Clin Nutr. 2011;94(6 Suppl):1933s–8s.
5. Cawley J, Meyerhoefer C. The medical care costs of obesity: an instrumental variables approach. J Health Econ. 2012;31(1):219–30.
6. McAllister EJ, Dhurandhar NV, Keith SW, Aronne LJ, Barger J, Baskin M, et al. Ten putative contributors to the obesity epidemic. Crit Rev Food Sci Nutr. 2009;49(10):868–913.
7. Gonzalez-Muniesa P, Martinez-Gonzalez MA, Hu FB, Despres JP, Matsuzawa Y, Loos RJF, et al. Obesity. Nat Rev Dis Primers. 2017;3:17034.
8. Campbell Am LV. Genetics of obesity. Aust Fam Physician. 2017;46(7):456–9.
9. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature. 2015;518(7538):197–206.
10. Aguilera CM, Olza J, Gil A. Genetic susceptibility to obesity and metabolic syndrome in childhood. Nutricion hospitalaria. 2013;28(Suppl 5):44–55.
11. Milagro FI, Moreno-Aliaga MJ, Martinez JA. FTO Obesity variant and adipocyte Browning in humans. N Engl J Med. 2016;374(2):190–1.
12. Serretti A, Mandelli L. Antidepressants and body weight: a comprehensive review and meta-analysis. J Clin Psychiatry. 2010;71(10):1259–72.
13. Verhaegen AA, Van Gaal LF. Drug-induced obesity and its metabolic consequences: a review with a focus on mechanisms and possible therapeutic options. J Endocrinol Investig. 2017;40:1165–74.
14. McCloughen A, Foster K. Weight gain associated with taking psychotropic medication: an integrative review. Int J Ment Health Nurs. 2011;20(3):202–22.
15. Medici V, McClave SA, Miller KR. Common medications which Lead to unintended alterations in weight gain or organ lipotoxicity. Curr Gastroenterol Rep. 2016;18(1):2.
16. Christ-Crain M, Kola B, Lolli F, Fekete C, Seboek D, Wittmann G, et al. AMP-activated protein kinase mediates glucocorticoid-induced metabolic changes: a novel mechanism in Cushing's syndrome. FASEB J. 2008;22(6):1672–83.
17. Xu MY, Cao B, Wang DF, Guo JH, Chen KL, Shi M, et al. Human adenovirus 36 infection increased the risk of obesity: a meta-analysis update. Medicine. 2015;94(51):e2357.
18. Shang Q, Wang H, Song Y, Wei L, Lavebratt C, Zhang F, et al. Serological data analyses show that adenovirus 36 infection is associated with obesity: a meta-analysis involving 5739 subjects. Obesity (Silver Spring). 2014;22(3):895–900.
19. Hainer V, Zamrazilova H, Kunesova M, Bendlova B, Aldhoon-Hainerova I. Obesity and infection: reciprocal causality. Physiol Res. 2015;64(Suppl 2):S105–19.
20. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013;341(6150):1241214.
21. Li J, Riaz Rajoka MS, Shao D, Jiang C, Jin M, Huang Q, et al. Strategies to increase the efficacy of using gut microbiota for the modulation of obesity. Obes Rev. 2017;18:1260–71.
22. Korpela K, de Vos WM. Antibiotic use in childhood alters the gut microbiota and predisposes to overweight. Microb Cell. 2016;3(7):296–8.
23. Mbakwa CA, Penders J, Savelkoul PH, Thijs C, Dagnelie PC, Mommers M, et al. Gut colonization with methanobrevibacter smithii is associated with childhood weight development. Obesity (Silver Spring). 2015;23(12):2508–16.

24. Kim J, Peterson KE, Scanlon KS, Fitzmaurice GM, Must A, Oken E, et al. Trends in overweight from 1980 through 2001 among preschool-aged children enrolled in a health maintenance organization. Obesity (Silver Spring). 2006;14(7):1107–12.
25. Lustig RH. The neuroendocrinology of obesity. Endocrinol Metab Clin N Am. 2001;30(3):765–85.
26. Brown RE, Sharma AM, Ardern CI, Mirdamadi P, Mirdamadi P, Kuk JL. Secular differences in the association between caloric intake, macronutrient intake, and physical activity with obesity. Obes Res Clin Pract. 2016;10(3):243–55.
27. Nadal A. Obesity fat from plastics? Linking bisphenol A exposure and obesity. Nat Rev Endocrinol. 2012;9:9–10.
28. Darbre PD. Endocrine disruptors and obesity. Curr Obes Rep. 2017;6:18–27.
29. Wang Y, Hollis-Hansen K, Ren X, Qiu Y, Qu W. Do environmental pollutants increase obesity risk in humans? Obes Rev. 2016;17(12):1179–97.
30. Heindel JJ, Blumberg B. Environmental obesogens: mechanisms and controversies. Annu Rev Pharmacol Toxicol. 2018;59:89–106.
31. Heindel JJ, Blumberg B, Cave M, Machtinger R, Mantovani A, Mendez MA, et al. Metabolism disrupting chemicals and metabolic disorders. Reprod Toxicol. 2017;68:3–33.
32. Le Magueresse-Battistoni B, Labaronne E, Vidal H, Naville D. Endocrine disrupting chemicals in mixture and obesity, diabetes and related metabolic disorders. World J Biol Chem. 2017;8(2):108–19.
33. Nappi F, Barrea L, Di Somma C, Savanelli MC, Muscogiuri G, Orio F, et al. Endocrine aspects of environmental "Obesogen" pollutants. Int J Environ Res Public Health. 2016;13(8):765.
34. Veiga-Lopez A, Pu Y, Gingrich J, Padmanabhan V. Obesogenic endocrine disrupting chemicals: identifying knowledge gaps. Trends Endocrinol Metab. 2018;29(9):607–25.
35. Heindel JJ, Balbus J, Birnbaum L, Brune-Drisse MN, Grandjean P, Gray K, et al. Developmental origins of health and disease: integrating environmental influences. Endocrinology. 2015;156(10):3416–21.
36. Eriksson JG. Developmental origins of health and disease - from a small body size at birth to epigenetics. Ann Med. 2016;48(6):456–67.
37. Regnier SM, El-Hashani E, Kamau W, Zhang X, Massad NL, Sargis RM. Tributyltin differentially promotes development of a phenotypically distinct adipocyte. Obesity (Silver Spring). 2015;23(9):1864–71.
38. Chamorro-Garcia R, Diaz-Castillo C, Shoucri BM, Kach H, Leavitt R, Shioda T, et al. Ancestral perinatal obesogen exposure results in a transgenerational thrifty phenotype in mice. Nat Commun. 2017;8(1):2012.
39. Shoucri BM, Hung VT, Chamorro-Garcia R, Shioda T, Blumberg B. Retinoid X receptor activation during adipogenesis of female mesenchymal stem cells programs a dysfunctional adipocyte. Endocrinology. 2018;159:2863–83.
40. Ariemma F, D'Esposito V, Liguoro D, Oriente F, Cabaro S, Liotti A, et al. Low-dose Bisphenol-A impairs Adipogenesis and generates dysfunctional 3T3-L1 adipocytes. PLoS One. 2016;11(3):e0150762.
41. Heindel JJ, Skalla LA, Joubert BR, Dilworth CH, Gray KA. Review of developmental origins of health and disease publications in environmental epidemiology. Reprod Toxicol. 2017;68:34–48.
42. Loche E, Ozanne SE. Non-genetic transmission of obesity: It's in your Epigenes. Trends Endocrinol Metab. 2016;27(6):349–50.
43. Kappil M, Chen J. Environmental exposures in utero and microRNA. Curr Opin Pediatr. 2014;26(2):243–51.
44. Braun JM. Pre-conception susceptibility to endocrine disruptors. Nat Rev Endocrinol. 2018;14:505–6.
45. Grandjean P, Barouki R, Bellinger DC, Casteleyn L, Chadwick LH, Cordier S, et al. Lifelong implications of developmental exposure to environmental stressors: new perspectives. Endocrinology. 2015;156(10):3408–15.

46. Godfrey KM, Costello PM, Lillycrop KA. Development, epigenetics and metabolic programming. Nestle Nutr Inst Workshop Ser. 2016;85:71–80.
47. Hanson MA, Gluckman PD. Developmental origins of health and disease: global public health implications. Best Pract Res Clin Obstet Gynaecol. 2014;29:24–31.
48. Tournaire M, Epelboin S, Devouche E, Viot G, Le Bidois J, Cabau A, et al. Adverse health effects in children of women exposed in utero to diethylstilbestrol (DES). Therapie. 2016;71(4):395–404.
49. Newbold RR. Prenatal exposure to diethylstilbestrol (DES). Fertil Steril. 2008;89(2):e55–e6.
50. Grun F. Endocrine-disrupting organotin compounds are potent inducers of adipogenesis in vertebrates. Mol Endocrinol. 2006;20:2141–55.
51. Baillie-Hamilton PF. Chemical toxins: a hypothesis to explain the global obesity epidemic. J Altern Complement Med. 2002;8(2):185–92.
52. Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, vom Saal FS. Exposure to bisphenol A advances puberty. Nature. 1999;401(6755):763–4.
53. Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect. 2001;109(7):675–80.
54. Swedenborg E, Ruegg J, Makela S, Pongratz I. Endocrine disruptive chemicals: mechanisms of action and involvement in metabolic disorders. J Mol Endocrinol. 2009;43(1):1–10.
55. La Merrill M, Birnbaum LS. Childhood obesity and environmental chemicals. Mt Sinai J Med. 2011;78(1):22–48.
56. Braun JM. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nat Rev Endocrinol. 2017;13(3):161–73.
57. Oken E, Levitan EB, Gillman MW. Maternal smoking during pregnancy and child overweight: systematic review and meta-analysis. Int J Obes. 2007;32(2):201–10.
58. Behl M, Rao D, Aagaard K, Davidson TL, Levin ED, Slotkin TA, et al. Evaluation of the association between maternal smoking, childhood obesity, and metabolic disorders: a national toxicology program workshop review. Environ Health Perspect. 2013;121(2):170–80.
59. Mendez MA, Torrent M, Ferrer C, Ribas-Fito N, Sunyer J. Maternal smoking very early in pregnancy is related to child overweight at age 5–7 years. Am J Clin Nutr. 2008;87(6):1906–13.
60. Somm E, Schwitzgebel VM, Vauthay DM, Camm EJ, Chen CY, Giacobino JP, et al. Prenatal nicotine exposure alters early pancreatic islet and adipose tissue development with consequences on the control of body weight and glucose metabolism later in life. Endocrinology. 2008;149(12):6289–99.
61. Fan J, W-X Z, Y-S R, J-L X, F-F W, Zhang L, et al. Perinatal nicotine exposure increases Obesity susceptibility in adult male rat offspring by altering early adipogenesis. Endocrinology. 2016;157(11):4276–86.
62. Bourez S, Le Lay S, Van den Daelen C, Louis C, Larondelle Y, Thomé J-P, et al. Accumulation of polychlorinated biphenyls in adipocytes: selective targeting to lipid droplets and role of Caveolin-1. PLoS One. 2012;7(2):e31834.
63. La Merrill M, Emond C, Kim MJ, Antignac J-P, Le Bizec B, Clément K, et al. Toxicological function of adipose tissue: focus on persistent organic pollutants. Environ Health Perspect. 2013;121(2):162–9.
64. Lee DH, Porta M, Jacobs DR Jr, Vandenberg LN. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. Endocr Rev. 2014;35(4):557–601.
65. Ruzzin J, Petersen R, Meugnier E, Madsen L, Lock E-J, Lillefosse H, et al. Persistent organic pollutant exposure leads to insulin resistance syndrome. Environ Health Perspect. 2010;118(4):465–71.
66. Ibrahim MM, Fjære E, Lock E-J, Naville D, Amlund H, Meugnier E, et al. Chronic consumption of farmed Salmon containing persistent organic pollutants causes insulin resistance and Obesity in mice. PLoS One. 2011;6(9):e25170.

67. Hong NS, Kim KS, Lee IK, Lind PM, Lind L, Jacobs DR, et al. The association between obesity and mortality in the elderly differs by serum concentrations of persistent organic pollutants: a possible explanation for the obesity paradox. Int J Obes. 2012;36(9):1170–5.
68. Lee YM, Kim KS, Jacobs DR Jr, Lee DH. Persistent organic pollutants in adipose tissue should be considered in obesity research. Obes Rev. 2017;18(2):129–39.
69. Cheikh Rouhou M, Karelis AD, St-Pierre DH, Lamontagne L. Adverse effects of weight loss: are persistent organic pollutants a potential culprit? Diabetes Metab. 2016;42:215–23.
70. Eskenazi B, Chevrier J, Rosas LG, Anderson HA, Bornman MS, Bouwman H, et al. The Pine River statement: human health consequences of DDT use. Environ Health Perspect. 2009;117(9):1359–67.
71. Hsu W-W, Rose Osuch J, Todem D, Taffe B, O'Keefe M, Adera S, et al. DDE and PCB serum concentration in maternal blood and their adult female offspring. Environ Res. 2014;132:384–90.
72. Karmaus W, Osuch JR, Eneli I, Mudd LM, Zhang J, Mikucki D, et al. Maternal levels of dichlorodiphenyl-dichloroethylene (DDE) may increase weight and body mass index in adult female offspring. Occup Environ Med. 2009;66(3):143–9.
73. Mendez MA, Garcia-Esteban R, Guxens M, Vrijheid M, Kogevinas M, Goni F, et al. Prenatal organochlorine compound exposure, rapid weight gain, and overweight in infancy. Environ Health Perspect. 2011;119(2):272–8.
74. Delvaux I, Van Cauwenberghe J, Den Hond E, Schoeters G, Govarts E, Nelen V, et al. Prenatal exposure to environmental contaminants and body composition at age 7-9 years. Environ Res. 2014;132:24–32.
75. Warner M, Wesselink A, Harley KG, Bradman A, Kogut K, Eskenazi B. Prenatal exposure to dichlorodiphenyltrichloroethane and obesity at 9 years of age in the CHAMACOS study cohort. Am J Epidemiol. 2014;179(11):1312–22.
76. Agay-Shay K, Martinez D, Valvi D, Garcia-Esteban R, Basagana X, Robinson O, et al. Exposure to endocrine-disrupting chemicals during pregnancy and weight at 7 years of age: a multi-pollutant approach. Environ Health Perspect. 2015;123(10):1030–7.
77. Iszatt N, Stigum H, Verner MA, White RA, Govarts E, Murinova LP, et al. Prenatal and postnatal exposure to persistent organic pollutants and infant growth: a pooled analysis of seven European birth cohorts. Environ Health Perspect. 2015;123(7):730–6.
78. La Merrill M, Karey E, Moshier E, Lindtner C, La Frano MR, Newman JW, et al. Perinatal exposure of mice to the pesticide DDT impairs energy expenditure and metabolism in adult female offspring. PLoS One. 2014;9(7):e103337.
79. Ibrahim MM, Fjære E, Lock E-J, Frøyland L, Jessen N, Lund S, et al. Metabolic impacts of high dietary exposure to persistent organic pollutants in mice. Toxicol Lett. 2012;215:8–15.
80. Moreno-Aliaga MJ, Matsumura F. Effects of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (p,p′-DDT) on 3T3-L1 and 3T3-F442A adipocyte differentiation. Biochem Pharmacol. 2002;63(5):997–1007.
81. Skinner MK, Manikkam M, Tracey R, Guerrero-Bosagna C, Haque M, Nilsson EE. Ancestral dichlorodiphenyltrichloroethane (DDT) exposure promotes epigenetic transgenerational inheritance of obesity. BMC Med. 2013;11:228.
82. Buck RC, Franklin J, Berger U, Conder JM, Cousins IT, de Voogt P, et al. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr Environ Assess Manag. 2011;7(4):513–41.
83. Lam J, Koustas E, Sutton P, Johnson PI, Atchley DS, Sen S, et al. The navigation guide - evidence-based medicine meets environmental health: integration of animal and human evidence for PFOA effects on fetal growth. Environ Health Perspect. 2014;122(10):1040–51.
84. Joca L, Sacks JD, Moore D, Lee JS, Sams Ii R, Cowden J. Systematic review of differential inorganic arsenic exposure in minority, low-income, and indigenous populations in the United States. Environ Int. 2016;92–93:707–15.

85. Johnson PI, Koustas E, Vesterinen HM, Sutton P, Atchley DS, Kim AN, et al. Application of the navigation guide systematic review methodology to the evidence for developmental and reproductive toxicity of Triclosan. Environ Int. 2016;92-93:716–28.
86. Liu P, Yang F, Wang Y, Yuan Z. Perfluorooctanoic acid (PFOA) exposure in early life increases risk of childhood adiposity: a meta-analysis of prospective cohort studies. Int J Environ Res Public Health. 2018;15(10):E2070.
87. Halldorsson TI, Rytter D, Haug LS, Bech BH, Danielsen I, Becher G, et al. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ Health Perspect. 2012;120:668–73.
88. Hines EP, White SS, Stanko JP, Gibbs-Flournoy EA, Lau C, Fenton SE. Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: low doses induce elevated serum leptin and insulin, and overweight in mid-life. Mol Cell Endocrinol. 2009;304(1–2):97–105.
89. Qi W, Clark JM, Timme-Laragy AR, Park Y. Perfluorobutanesulfonic acid (PFBS) potentiates adipogenesis of 3T3-L1 adipocytes. Food Chem Toxicol. 2018;120:340–5.
90. Sant KE, Venezia OL, Sinno PP, Timme-Laragy AR. Perfluorobutanesulfonic acid disrupts pancreatic organogenesis and regulation of lipid metabolism in the zebrafish, Danio rerio. Toxicol Sci. 2018;167:258–68.
91. Vanden Heuvel JP, Thompson JT, Frame SR, Gillies PJ. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. Toxicol Sci. 2006;92(2):476–89.
92. Arsenescu V, Arsenescu RI, King V, Swanson H, Cassis LA. Polychlorinated Biphenyl-77 induces adipocyte differentiation and Proinflammatory Adipokines and promotes Obesity and atherosclerosis. Environ Health Perspect. 2008;116(6):761–8.
93. Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB, et al. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation. 2009;119(4):538–46.
94. Xu X, Yavar Z, Verdin M, Ying Z, Mihai G, Kampfrath T, et al. Effect of early particulate air pollution exposure on obesity in mice: role of p47phox. Arterioscler Thromb Vasc Biol. 2010;30(12):2518–27.
95. Bolton JL, Auten RL, Bilbo SD. Prenatal air pollution exposure induces sexually dimorphic fetal programming of metabolic and neuroinflammatory outcomes in adult offspring. Brain Behav Immun. 2014;37:30–44.
96. Weldy CS, Liu Y, Liggitt HD, Chin MT. In utero exposure to diesel exhaust air pollution promotes adverse intrauterine conditions, resulting in weight gain, altered blood pressure, and increased susceptibility to heart failure in adult mice. PLoS One. 2014;9(2):e88582.
97. Ortiz L, Nakamura B, Li X, Blumberg B, Luderer U. In utero exposure to benzo[a]pyrene increases adiposity and causes hepatic steatosis in female mice, and glutathione deficiency is protective. Toxicol Lett. 2013;223(2):260–7.
98. Irigaray P, Ogier V, Jacquenet S, Notet V, Sibille P, Mejean L, et al. Benzo[a]pyrene impairs beta-adrenergic stimulation of adipose tissue lipolysis and causes weight gain in mice. A novel molecular mechanism of toxicity for a common food pollutant. FEBS J. 2006;273(7):1362–72.
99. Wei Y, Zhang JJ, Li Z, Gow A, Chung KF, Hu M, et al. Chronic exposure to air pollution particles increases the risk of obesity and metabolic syndrome: findings from a natural experiment in Beijing. FASEB J. 2016;30:2115–22.
100. Rundle A, Hoepner L, Hassoun A, Oberfield S, Freyer G, Holmes D, et al. Association of childhood obesity with maternal exposure to ambient air polycyclic aromatic hydrocarbons during pregnancy. Am J Epidemiol. 2012;175:1163–72.
101. McConnell R, Gilliland FD, Goran M, Allayee H, Hricko A, Mittelman S. Does near-roadway air pollution contribute to childhood obesity? Pediatr Obes. 2015;11:1–3.

102. Braun JM, Gray K. Challenges to studying the health effects of early life environmental chemical exposures on children's health. PLoS Biol. 2017;15(12):e2002800.
103. vom Saal FS, Nagel SC, Coe BL, Angle BM, Taylor JA. The estrogenic endocrine disrupting chemical bisphenol A (BPA) and obesity. Mol Cell Endocrinol. 2012;354:74–84.
104. Vandenberg LN. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139.
105. Calafat AM. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003–2004. Environ Health Perspect. 2008;116(1):39.
106. Babu S, Uppu SN, Martin B, Agu OA, Uppu RM. Unusually high levels of bisphenol a (BPA) in thermal paper cash register receipts (CRs): development and application of a robust LC-UV method to quantify BPA in CRs. Toxicol Mech Methods. 2015;25(5):410–6.
107. Legeay S, Faure S. Is bisphenol A an environmental obesogen? Fund Clin Pharmacol. 2017;31(6):594–609.
108. Boucher JG, Gagné R, Rowan-Carroll A, Boudreau A, Yauk CL, Atlas E. Bisphenol a and Bisphenol S induce distinct transcriptional profiles in differentiating human primary Preadipocytes. PLoS One. 2016;11(9):e0163318.
109. Masuno H, Iwanami J, Kidani T, Sakayama K, Honda K. Bisphenol a accelerates terminal differentiation of 3T3-L1 cells into adipocytes through the phosphatidylinositol 3-kinase pathway. Toxicol Sci. 2005;84(2):319–27.
110. Boucher JG, Boudreau A, Atlas E. Bisphenol A induces differentiation of human preadipocytes in the absence of glucocorticoid and is inhibited by an estrogen-receptor antagonist. Nutr Diabetes. 2014;4(1):e102.
111. Bouret S, Levin BE, Ozanne SE. Gene-environment interactions controlling energy and glucose homeostasis and the developmental origins of obesity. Physiol Rev. 2015;95(1):47–82.
112. Boucher JG, Boudreau A, Ahmed S, Atlas E. In vitro effects of Bisphenol A beta-D-glucuronide (BPA-G) on adipogenesis in human and murine preadipocytes. Environ Health Perspect. 2015;123(12):1287–93.
113. Boucher JG, Ahmed S, Atlas E. Bisphenol S induces adipogenesis in primary human preadipocytes from female donors. Endocrinology. 2016;157(4):1397–407.
114. Rubin BS, Paranjpe M, DaFonte T, Schaeberle C, Soto AM, Obin M, et al. 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. 2017;68:130–44.
115. Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, et al. Perinatal exposure to bisphenol a alters early adipogenesis in the rat. Environ Health Perspect. 2009;117(10):1549–55.
116. Angle BM, Do RP, Ponzi D, Stahlhut RW, Drury BE, Nagel SC, et al. 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. 2013;42:256–68. https://doi.org/10.1016/j.reprotox.2013.07.017.
117. Desai M, Ferrini MG, Han G, Jellyman JK, Ross MG. In vivo maternal and in vitro BPA exposure effects on hypothalamic neurogenesis and appetite regulators. Environ Res. 2018;164:45–52.
118. Johnson SA, Painter MS, Javurek AB, Ellersieck MR, Wiedmeyer CE, Thyfault JP, et al. Sex-dependent effects of developmental exposure to bisphenol A and ethinyl estradiol on metabolic parameters and voluntary physical activity. J Dev Orig Health Dis. 2015;6:1–14.
119. Hoepner LA, Whyatt RM, Widen EM, Hassoun A, Oberfield SE, Mueller NT, et al. Bisphenol A and adiposity in an Inner-City birth cohort. Environ Health Perspect. 2016;124:1644–50.
120. Vafeiadi M, Georgiou V, Chalkiadaki G, Rantakokko P, Kiviranta H, Karachaliou M, et al. 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. 2015;123(10):1015–21.

121. Harley KG, Aguilar Schall R, Chevrier J, Tyler K, Aguirre H, Bradman A, et al. Prenatal and postnatal bisphenol A exposure and body mass index in childhood in the CHAMACOS cohort. Environ Health Perspect. 2013;121(4):514–20.
122. Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. Int J Hyg Environ Health. 2007;210(5):623–34.
123. Woodruff TJ, Zota AR, Schwartz JM. Environmental chemicals in pregnant women in the United States: NHANES 2003–2004. Environ Health Perspect. 2011;119(6):878–85.
124. Hao C, Cheng X, Guo J, Xia H, Ma X. Perinatal exposure to diethyl-hexyl-phthalate induces obesity in mice. Front Biosci (Elite Ed). 2013;5:725–33.
125. Hao C, Cheng X, Xia H, Ma X. The endocrine disruptor mono-(2-ethylhexyl) phthalate promotes adipocyte differentiation and induces obesity in mice. Biosci Rep. 2012;32(6):619–29.
126. Schmidt J-S, Schaedlich K, Fiandanese N, Pocar P, Fischer B. Di(2-ethylhexyl) phthalate (DEHP) impairs female fertility and promotes adipogenesis in C3H/N mice. Environ Health Perspect. 2012;120:1123–9.
127. Gu H, Liu Y, Wang W, Ding L, Teng W, Liu L. 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. 2016;12(6):3806–12.
128. Feige JN, Gelman L, Rossi D, Zoete V, Metivier R, Tudor C, et al. The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor gamma modulator that promotes adipogenesis. J Biol Chem. 2007;282(26):19152–66.
129. Hurst CH, Waxman DJ. Activation of PPARalpha and PPARgamma by environmental phthalate monoesters. Toxicol Sci. 2003;74(2):297–308.
130. Biemann R, Fischer B, Navarrete Santos A. Adipogenic effects of a combination of the endocrine-disrupting compounds bisphenol A, diethylhexylphthalate, and tributyltin. Obes Facts. 2014;7(1):48–56.
131. Chiu CY, Sun SC, Chiang CK, Wang CC, Chan DC, Chen HJ, et al. Plasticizer di(2-ethylhexyl)phthalate interferes with osteoblastogenesis and adipogenesis in a mouse model. J Orthop Res. 2018;36(4):1124–34.
132. Howdeshell KL, Wilson VS, Furr J, Lambright CR, Rider CV, Blystone CR, et al. A mixture of five phthalate esters inhibits fetal testicular testosterone production in the sprague-dawley rat in a cumulative, dose-additive manner. Toxicol Sci. 2008;105(1):153–65.
133. Kim M, Jeong JS, Kim H, Hwang S, Park IH, Lee BC, et al. Low dose exposure to Di-2-Ethylhexylphthalate in juvenile rats alters the expression of genes related with thyroid hormone regulation. Biomol Ther. 2018;26(5):512–9.
134. Johns LE, Ferguson KK, Soldin OP, Cantonwine DE, Rivera-González LO, Del Toro LVA, et al. Urinary phthalate metabolites in relation to maternal serum thyroid and sex hormone levels during pregnancy: a longitudinal analysis. Reprod Biol Endocrinol. 2015;13:4.
135. Grimaldi M, Boulahtouf A, Delfosse V, Thouennon E, Bourguet W, Balaguer P. Reporter cell lines for the characterization of the interactions between human nuclear receptors and endocrine disruptors. Front Endocrinol. 2015;6:62.
136. Ferguson KK, McElrath TF, Chen Y-H, Mukherjee B, Meeker JD. Urinary phthalate metabolites and biomarkers of oxidative stress in pregnant women: a repeated measures analysis. Environ Health Perspect. 2015;123(3):210–6.
137. Yavasoglu NU, Koksal C, Dagdeviren M, Aktug H, Yavasoglu A. Induction of oxidative stress and histological changes in liver by subacute doses of butyl cyclohexyl phthalate. Environ Toxicol. 2014;29(3):345–53.
138. Lin Y, Wei J, Li Y, Chen J, Zhou Z, Song L, et al. 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 Endocrinol Metab. 2011;301(3):E527–38.
139. Lv Z, Cheng J, Huang S, Zhang Y, Wu S, Qiu Y, et al. DEHP induces obesity and hypothyroidism through both central and peripheral pathways in C3H/He mice. Obesity (Silver Spring). 2016;24(2):368–78.

140. Wassenaar PNH, Legler J. Systematic review and meta-analysis of early life exposure to di(2-ethylhexyl) phthalate and obesity related outcomes in rodents. Chemosphere. 2017;188:174–81.
141. Janesick AS, Blumberg B. Obesogens: an emerging threat to public health. Am J Obstet Gynecol. 2016;214:559–65.
142. Watt J, Schlezinger JJ. Structurally-diverse, PPARgamma-activating environmental toxicants induce adipogenesis and suppress osteogenesis in bone marrow mesenchymal stromal cells. Toxicology. 2015;331:66–77.
143. Li X, Ycaza J, Blumberg B. The environmental obesogen tributyltin chloride acts via peroxisome proliferator activated receptor gamma to induce adipogenesis in murine 3T3-L1 preadipocytes. J Steroid Biochem Mol Biol. 2011;127(1–2):9–15.
144. Zuo Z, Chen S, Wu T, Zhang J, Su Y, Chen Y, et al. Tributyltin causes obesity and hepatic steatosis in male mice. Environ Toxicol. 2011;26(1):79–85.
145. Pereira-Fernandes A, Demaegdt H, Vandermeiren K, Hectors TL, Jorens PG, Blust R, et al. Evaluation of a screening system for obesogenic compounds: screening of endocrine disrupting compounds and evaluation of the PPAR dependency of the effect. PLoS One. 2013;8(10):e77481.
146. Kim S, Li A, Monti S, Schlezinger JJ. Tributyltin induces a transcriptional response without a brite adipocyte signature in adipocyte models. Arch Toxicol. 2018;92:2859–74.
147. Chamorro-Garcia R. Transgenerational inheritance of increased fat depot size, stem cell reprogramming, and hepatic steatosis elicited by prenatal obesogen tributyltin in mice. Environ Health Perspect. 2013;121:359–66.
148. Hu P, Chen X, Whitener RJ, Boder ET, Jones JO, Porollo A, et al. Effects of parabens on adipocyte differentiation. Toxicol Sci. 2013;131(1):56–70.
149. Hines EP, Mendola P, von Ehrenstein OS, Ye X, Calafat AM, Fenton SE. Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women. Reprod Toxicol. 2015;54:120–8.
150. Calafat AM, Ye X, Wong LY, Bishop AM, Needham LL. Urinary concentrations of four parabens in the U.S. population: NHANES 2005–2006. Environ Health Perspect. 2010;118(5):679–85.
151. Hu P, Kennedy RC, Chen X, Zhang J, Shen CL, Chen J, et al. Differential effects on adiposity and serum marker of bone formation by post-weaning exposure to methylparaben and butylparaben. Environ Sci Pollut Res Int. 2016;23(21):21957–68.
152. Hu P, Overby H, Heal E, Wang S, Chen J, Shen CL, et al. Methylparaben and butylparaben alter multipotent mesenchymal stem cell fates towards adipocyte lineage. Toxicol Appl Pharmacol. 2017;329:48–57.
153. Wu C, Huo W, Li Y, Zhang B, Wan Y, Zheng T, et al. Maternal urinary paraben levels and offspring size at birth from a Chinese birth cohort. Chemosphere. 2017;172:29–36.
154. Philippat C, Botton J, Calafat AM, Ye X, Charles M-A, Slama R, et al. Prenatal exposure to phenols and growth in boys. Epidimiology. 2014;25(5):625–35.
155. Yueh M-F, Tukey RH. Triclosan: a widespread environmental toxicant with many biological effects. Annu Rev Pharmacol Toxicol. 2016;56:251–72.
156. Johnson PI, Koustas E, Vesterinen HM, Sutton P, Atchley DS, Kim AN, et al. Application of the navigation guide systematic review methodology to the evidence for developmental and reproductive toxicity of triclosan. Environ Int. 2016;92?93.
157. Arbuckle TE, Weiss L, Fisher M, Hauser R, Dumas P, Berube R, et al. Maternal and infant exposure to environmental phenols as measured in multiple biological matrices. Sci Total Environ. 2015;508:575–84.
158. Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Urinary concentrations of triclosan in the U.S. population: 2003–2004. Environ Health Perspect. 2008;116(3):303–7.
159. Buckley JP, Herring AH, Wolff MS, Calafat AM, Engel SM. Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's environmental health study. Environ Int. 2016;91:350–6.

160. Hao CJ, Cheng XJ, Xia HF, Ma X. The endocrine disruptor 4-nonylphenol promotes adipocyte differentiation and induces obesity in mice. Cell Physiol Biochem. 2012;30(2):382–94.
161. Lim S, Ahn SY, Song IC, Chung MH, Jang HC, Park KS, et al. Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance. PLoS One. 2009;4(4):e5186.
162. Li X, Pham HT, Janesick AS, Blumberg B. Triflumizole is an obesogen in mice that acts through peroxisome proliferator activated receptor gamma (PPARgamma). Environ Health Perspect. 2012;120(12):1720–6.
163. Xiao X, Qi W, Clark JM, Park Y. Permethrin potentiates adipogenesis via intracellular calcium and endoplasmic reticulum stress-mediated mechanisms in 3T3-L1 adipocytes. Food Chem Toxicol. 2017;109(Pt 1):123–9.
164. Shen P, Hsieh TH, Yue Y, Sun Q, Clark JM, Park Y. Deltamethrin increases the fat accumulation in 3T3-L1 adipocytes and *Caenorhabditis elegans*. Food Chem Toxicol. 2017;101:149–56.
165. Sun Q, Qi W, Yang JJ, Yoon KS, Clark JM, Park Y. Fipronil promotes adipogenesis via AMPKalpha-mediated pathway in 3T3-L1 adipocytes. Food Chem Toxicol. 2016;92:217–23.
166. Leasure JL, Giddabasappa A, Chaney S, Johnson JE, Pothakos K, Lau YS, et al. Low-level human equivalent gestational Lead exposure produces sex-specific motor and coordination abnormalities and late-onset obesity in year-old mice. Environ Health Perspect. 2008;116(3):355–61.
167. Bunyan J, Murrell EA, Shah PP. The induction of obesity in rodents by means of monosodium glutamate. Br J Nutr. 1976;35(1):25–39.
168. Tappy L. Fructose-containing caloric sweeteners as a cause of obesity and metabolic disorders. J Exp Biol. 2018;221(Pt Suppl 1):jeb164202.
169. Suvorov A, Battista MC, Takser L. Perinatal exposure to low-dose 2,2′,4,4′-tetrabromodiphenyl ether affects growth in rat offspring: what is the role of IGF-1? Toxicology. 2009;260(1–3):126–31.
170. Pillai HK, Fang M, Beglov D, Kozakov D, Vajda S, Stapleton HM, et al. Ligand binding and activation of PPARgamma by Firemaster(R) 550: effects on adipogenesis and osteogenesis in vitro. Environ Health Perspect. 2014;122(11):1225–32.
171. Woeller CF, Flores E, Pollock SJ, Phipps RP. Editor's highlight: Thy1 (CD90) expression is reduced by the environmental chemical Tetrabromobisphenol-A to promote adipogenesis through induction of microRNA-103. Toxicol Sci. 2017;157(2):305–19.
172. Chamorro-Garcia R, Kirchner S, Li X, Janesick A, Casey SC, Chow C, et al. Bisphenol A diglycidyl ether induces adipogenic differentiation of multipotent stromal stem cells through a peroxisome proliferator-activated receptor gamma-independent mechanism. Environ Health Perspect. 2012;120(7):984–9.
173. Sargis RM, Johnson DN, Choudhury RA, Brady MJ. Environmental endocrine disruptors promote Adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. Obesity (Silver Spring). 2010;18(7):1283–8.
174. Park Y, Kim Y, Kim J, Yoon KS, Clark J, Lee J. Imidacloprid, a neonicotinoid insecticide, potentiates adipogenesis in 3T3-L1 adipocytes. J Agric Food Chem. 2013;61(1):255–9.
175. Fowler SPG. Low-calorie sweetener use and energy balance: results from experimental studies in animals, and large-scale prospective studies in humans. Physiol Behav. 2016;164:517–23.
176. Swithers SE. Artificial sweeteners are not the answer to childhood obesity. Appetite. 2015;93:85–90.
177. Fowler SP, Williams K, Resendez RG, Hunt KJ, Hazuda HP, Stern MP. Fueling the obesity epidemic? Artificially sweetened beverage use and Long-term weight gain. Obesity (Silver Spring). 2008;16(8):1894–900.
178. Boucher JG, Ahmed S, Atlas E, Bisphenol S. Induces adipogenesis in primary human preadipocytes from female donors. Endocrinology. 2016;157(4):1397–407.
179. Kassotis CD, Hoffman K, Stapleton HM. Characterization of Adipogenic activity of house dust extracts and semi-volatile indoor contaminants in 3T3-L1 cells. Environ Sci Technol. 2017;51:8735–45.

180. Skinner MK. A new kind of inheritance. Sci Am. 2014;311(2):44–51.
181. Skinner MK. What is an epigenetic transgenerational phenotype? F3 or F2. Reprod Toxicol. 2008;25(1):2–6.
182. Hanson MA, Skinner MK. Developmental origins of epigenetic transgenerational inheritance. Environ Epigen. 2016;2(1):dvw002.
183. Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. PLoS One. 2013;8(1):e55387.
184. Tracey R, Manikkam M, Guerrero-Bosagna C, Skinner MK. Hydrocarbons (jet fuel JP-8) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations. Reprod Toxicol. 2013;36:104–16.
185. Manikkam M, Tracey R, Guerrero-Bosagna C, Skinner MK. Dioxin (TCDD) induces epigenetic transgenerational inheritance of adult onset disease and sperm epimutations. PLoS One. 2012;7(9):e46249.
186. Fildes A, Charlton J, Rudisill C, Littlejohns P, Prevost AT, Gulliford MC. Probability of an obese person attaining normal body weight: cohort study using electronic health records. Am J Public Health. 2015;105:e1–6.
187. Lazzeretti L, Rotella F, Pala L, Rotella CM. Assessment of psychological predictors of weight loss: how and what for? World J Psychiatry. 2015;5(1):56–67.
188. Teixeira PJ, Carraça EV, Marques MM, Rutter H, Oppert J-M, De Bourdeaudhuij I, et al. Successful behavior change in obesity interventions in adults: a systematic review of self-regulation mediators. BMC Med. 2015;13:84.
189. Jiang X, Ma H, Wang Y, Liu Y. Early life factors and type 2 diabetes mellitus. J Diab Res. 2013;2013:485082.
190. Myers MG, Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: distinguishing cause from effect. Trends Endocrinol Metab. 2010;21(11):643–51.
191. Baik J-H. Dopamine signaling in food addiction: role of dopamine D2 receptors. BMB Rep. 2013;46(11):519–26.
192. Yu YH, Vasselli JR, Zhang Y, Mechanick JI, Korner J, Peterli R. Metabolic vs. hedonic obesity: a conceptual distinction and its clinical implications. Obes Rev. 2015;16(3):234–47.
193. Newbold RR, Padilla-Banks E, Snyder RJ, Jefferson WN. Perinatal exposure to environmental estrogens and the development of obesity. Mol Nutr Food Res. 2007;51(7):912–7.
194. Pestana D, Teixeira D, Meireles M, Marques C, Norberto S, Sa C, et al. Adipose tissue dysfunction as a central mechanism leading to dysmetabolic obesity triggered by chronic exposure to p,p'-DDE. Sci Rep. 2017;7(1):2738.
195. Sun Q, Xiao X, Kim Y, Kim D, Yoon KS, Clark JM, et al. Imidacloprid promotes high fat diet-induced adiposity and insulin resistance in male C57BL/6J mice. J Agric Food Chem. 2016;64(49):9293–306.
196. Chamorro-Garcia R, Diaz-Castillo C, Shoucri BM, Käch H, Leavitt R, Shioda T, et al. Ancestral perinatal obesogen exposure results in a transgenerational thrifty phenotype in mice. Nat Commun. 2017;8:2012.
197. De Long NE, Holloway AC. Early-life chemical exposures and risk of metabolic syndrome. Diabetes Metab Syndr Obes. 2017;10:101–9.
198. Mimoto MS, Nadal A, Sargis RM. Polluted pathways: mechanisms of metabolic disruption by endocrine disrupting chemicals. Curr Environ Health Rep. 2017;4(2):208–22.
199. Sun Q, Qi W, Xiao X, Yang SH, Kim D, Yoon KS, et al. Imidacloprid promotes high fat diet-induced adiposity in female C57BL/6J mice and enhances Adipogenesis in 3T3-L1 adipocytes via the AMPKalpha-mediated pathway. J Agric Food Chem. 2017;65(31):6572–81.
200. Regnier SM, Kirkley AG, Ye H, El-Hashani E, Zhang X, Neel BA, et al. Dietary exposure to the endocrine disruptor Tolylfluanid promotes global metabolic dysfunction in male mice. Endocrinology. 2014;156:896–910.

201. Lubrano C, Genovesi G, Specchia P, Costantini D, Mariani S, Petrangeli E, et al. Obesity and metabolic comorbidities: environmental diseases? Oxidative Med Cell Longev. 2013;2013:640673.
202. Bansal A, Henao-Mejia J, Simmons RA. Immune system: an emerging player in mediating effects of endocrine disruptors on metabolic health. Endocrinology. 2018;159(1):32–45.
203. Heindel JJ, Schug TT. The perfect storm for obesity. Obesity (Silver Spring). 2013;21(6):1079–80.
204. Sarker G, Berrens R, von Arx J, Pelczar P, Reik W, Wolfrum C, et al. Transgenerational transmission of hedonic behaviors and metabolic phenotypes induced by maternal overnutrition. Transl Psychiatry. 2018;8(1):195.
205. Heindel JJ, vom Saal FS, Blumberg B, Bovolin P, Calamandrei G, Ceresini G, et al. Parma consensus statement on metabolic disruptors. Environ Health. 2015;14:54.

Part III
Impact of Environmental Chemical Hazards on Human Development

Chapter 10
Impact of Air Pollution Hazards on Human Development

Eunhee Ha

Abstract Air pollutants like carbon monoxide (CO), ozone (O_3), nitrogen oxides (NO, NO_2, NO_x), lead (Pb), mercury (Hg), sulfur dioxide (SO_2), polycyclic aromatic hydrocarbons (PAHs), and particulate matter ($PM_{2.5}$ and PM_{10}) have known to exhibit harmful effects on different organs and systems in human body. Increasing exposure to air pollution in children is a global public health concern as children are extremely vulnerable to air pollution due to their dynamic growth. Under age five mortalities due to air pollution are increasing worldwide thus making it necessary to take prompt action on protecting children's environmental health. Air pollutants like PM_{10}, $PM_{2.5}$, SO_2, O_3, NO_2, CO are known to be strongly associated with adverse birth outcomes like low birth weight, preterm birth, and small for gestational age. Air pollution is found to be linked with neurocognitive development in children. NO_2 was found to be associated with psychomotor development in children, while $PM_{2.5}$ and PM_{10} were found to be associated with autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD). Studies have reported an association between air pollution especially PM and NO_2 and childhood obesity/insulin resistance. Thus, more advanced research is needed to implement effective strategies safeguarding children's health.

Keywords Air pollution · PM_{10} · $PM_{2.5}$ · SO_2 · O_3 · NO_2 · CO · Prenatal · Postnatal · Human development

E. Ha (✉)
Department of Occupational and Environment Medicine, College of Medicine, Ewha Womans University, Seoul, Republic of Korea
e-mail: eunheeha@ewha.ac.kr

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_10

10.1 Introduction

Children around the globe are exposed to air pollution. Of the global population, 92% live in areas with ambient air pollution levels that exceed World Health Organization (WHO) limits, including billions of children. Air pollution causes approximately 600,000 deaths of children under 5 years of age annually and increases the risk for respiratory infections, asthma, and adverse birth outcomes. Growing evidence also suggests that air pollution can adversely affect children's cognitive development, and early exposure might induce the development of chronic diseases in adulthood [1, 2]. Although historically air pollution has been thought of as a respiratory toxicant, recent evidence has broadened our understanding of its full range of effects. These associations may or may not be causal but clearly warrant additional study [3].

Ambient air pollution can result from the combustion of fossil fuels (including domestic heating, cooking and lighting, power generation, and motor vehicle exhaust), industrial processes, waste incineration, as well as natural processes (thunderstorms and volcanic eruptions) [training module]. Key ambient air pollutants include carbon monoxide (CO), ozone (O_3), nitrogen oxides (NO, NO_2, NO_x), lead (Pb), mercury (Hg), sulfur dioxide (SO_2), polycyclic aromatic hydrocarbons (PAHs), and particulate matter ($PM_{2.5}$ and PM_{10}) [1].

Def. Particulate Matter
Small particulate matter with a diameter of less than 10 microns is termed PM10, and its subset, PM2.5, are particles with a diameter of 2.5 microns or less, and both are widely used indicators of ambient air quality. The size of particulate matter determines where in the body the pollutant is deposited; particles larger than PM10 are typically filtered out through the nose while smaller particles can reach the lower airways. However, children typically breathe through their mouth, bypassing the nasal filtration mechanism, and these pollutants are able to penetrate deep into the child's lungs and the cardiovascular system [1].

The "WHO air quality guidelines" (Table 10.1) were proposed to help reduce the severe impact that air pollution can have on human health. The guidelines offer global guidance on the thresholds and limits for key air pollutants.

This chapter primarily reviews the impact of air pollution on infant mortality, fetal growth, and birth outcomes. Neurobehavioral diseases, such as autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) also are reviewed in this chapter as these diseases too have been linked to childhood, air pollution exposure. Conditions, such as obesity or insulin resistance, also are included in this review. These conditions are important to include as they have the potential to develop into metabolic syndrome or diabetes mellitus in later life.

Past and current research have proven that it is crucial to cover this matter, and more advanced research is needed. Unfortunately, humans cannot avoid the

Table 10.1 WHO air quality guideline values [1]

Pollutant	Common source of exposure	WHO guideline values
Particulate matter (PM) Fine particulate matter (PM2.5) Coarse particulate matter (PM10)	A direct source, such as construction sites, unpaved roads, fields, smokestacks or fires; secondary reactions of chemicals such as sulfur dioxide and nitrogen oxides of pollutants emitted from power plants, industries, and automobiles	10 μg/m^3 annual mean25 μg/m^3 24-h mean 20 μg/m^3 annual mean50 μg/m^3 24-h mean
Ozone (O_3)	Secondary pollutant formed by chemical reaction of volatile organic compounds (VOCs) and NO_x in the presence of sunlight	100 μg/m^3 8-h mean
Nitrogen dioxide (NO_2)	Combustion processes from heating, power generation, and engines in vehicles and ships	40 μg/m^3 annual mean 200 μg/m^3 1-h mean
Sulfur dioxide (SO_2)	Burning of sulfur-containing fossil fuels for domestic heating, power generation, and motor vehicles	20 μg/m^3 24-h mean 500 μg/m^3 10-min mean

negative impact of ambient and household air pollution since inhalation is a continuous activity that occurs in everyday life. Moreover, a breadth of research has provided evidence suggesting children, compared to adults, are uniquely susceptible to air pollution exposure as, is well understood, infants and children are undergoing critical and rapid cellular, system and organ growth, organization, and development.

Consequently, this chapter reviews the health effects of air pollution exposure on children including infant mortality, birth outcomes, neurocognitive development, and childhood obesity. This review includes relevant published studies, prioritizing systematic reviews, meta-analyses, and recent studies—primarily those published within the last 10 years. In addition, each section will present definitions of key health outcomes, biological mechanisms, and a brief summary.

10.2 Infant Mortality

10.2.1 Air Pollution Effects on Infant Mortality

Maternal exposure to air pollutants during pregnancy has been linked to infant mortality, which is defined below.

Def 2.1. Infant Mortality
Infant mortality is the death of an infant before his or her first birthday. The infant mortality rate is the number of infant deaths for every 1000 live births. In addition to giving us key information about maternal and infant health, the infant mortality rate is an important marker of the overall health of a society. In 2016, the infant mortality rate in the USA was 5.9 deaths per 1000 live births (https://www.cdc.gov/reproductivehealth/maternalinfanthealth/infantmortality.htm).

Numerous epidemiological studies have demonstrated the link between air pollution and adverse outcomes, including hospitalizations, emergency room visits, decreased lung function, increased risks of cardiovascular diseases, and mortality [4–7]. However, most of these studies are adult-focused and relatively few studies have examined the detrimental effects of air pollution on infant health.

Exposure to air pollution may result in increased risk of infant mortality because infants are possibly more susceptible to air pollution due to the immaturity of their lungs and immune system [8]. Therefore, a keen interest in preventing infant mortality attributable to air pollution is required. However, tentative conclusions from two systematic reviews suggest that although inferences on particulate matter and infant mortality due to respiratory causes are sufficient, the evidence of an association between particulate matter and infant mortality from other causes is still inconsistent and insufficient. More research is needed to clarify the link [9, 10]. As the systematic reviews were conducted in 2004 and 2005, an up-to-date review may be helpful for further identification of the association between particulate matter and infant mortality. Hence, to introduce the recent scientific research on this issue and summarize the evidence, included in this review are studies published after 2010 which investigated the effects of particulate matter on infant mortality.

Jung et al. [11] reported the effects of pre- and postnatal exposure to $PM_{2.5}$ on infant mortality using 2010–2015 retrospective birth cohort data obtained from the Statistics Korea. A total of 2,628,904 infants with 1509 deaths were identified. The mean $PM_{2.5}$ concentrations were calculated using CMAQ data for the following exposure periods: each trimester, gestation, postnatal period, and pre- and postnatal periods. The study indicated that a higher risk of infant mortality was associated with exposure to $PM_{2.5}$ during prenatal and postnatal periods.

Yorifuji et al. [12] analyzed the association between infant mortality and acute exposure to $PM_{2.5}$, SPM (suspended particulate matter), and $PM_{7–2.5}$ in Tokyo, Japan. The studied population included 2086 infants who died in the 23 urbanized wards of the Tokyo Metropolitan Government between January 2002 and December 2013. Infant mortality was further categorized by age at death (infant, neonatal, and post-neonatal mortality) and cause of death (cardiac diseases, respiratory diseases, perinatal circumstances, congenital and chromosomal abnormalities, and sudden infant death syndrome [SIDS]). This study reported the increased risks of infant mortality, post-neonatal mortality, and mortality due to respiratory diseases per a 10 μg/m^3 increase in $PM_{2.5}$. In addition, $PM_{7–2.5}$ was associated with a 21% increased risk of post-neonatal mortality. The risks of post-neonatal mortality and mortality due to respiratory diseases increased by 10% and 25%, respectively, for a 10 μg/m^3 increase in PM_7. The study also highlighted the elevated risks of infant and post-neonatal mortality even when $PM_{2.5}$ and SPM concentrations were below Japanese air quality guidelines.

Carbajal-Arroyo et al. [13] studied the effect of PM_{10} and O_3 on infant mortality in Mexico City, Mexico. Daily mortality data were obtained for infants under 1 years of age (1–11 months old) living in the 14 municipalities of Mexico City between January 1, 1997 and December 31, 2005. Infant mortality was stratified by cause of death (respiratory diseases) and socioeconomic status (high, medium, and low). PM_{10} was significantly associated with all-cause mortality with a one-day lag and a two-day lag by 5.5% and 6.6%, respectively, for a 38.7 μg/m^3 (interquartile range [IQR]) increase in PM_{10}. The risk of respiratory mortality increased by 9.8%

at a two-day lag with the same PM_{10} increase level. Cumulative exposure to PM_{10} from day zero to day two was found to be associated with an increased risk of infant mortality by 6.3% per IQR change. The effects of PM_{10} were assessed within the first and fourth quartiles of O_3 concentrations. The results showed elevated risks of infant mortality at lag 0, lag 1, lag 2, and lag 0–2 at the highest O_3 quartile ($\geq$130.5 ppb). When stratified by socioeconomic status, infant mortality and respiratory mortality significantly increased in the low SES group with respect to the concentration of PM_{10}. O_3 was also associated with respiratory mortality in the low SES group.

Son et al. [14] conducted a cohort study, examining the association between long-term exposure to TSP (total suspended particles), PM_{10}, $PM_{10-2.5}$, and $PM_{2.5}$ and infant mortality in South Korea. The studied population included 359,459 births with 225 deaths. Infants who died in the neonatal period (<28 days) were excluded from the analysis. Infant mortality was categorized by cause of death (all-cause, respiratory, and SIDS). The window of exposure included both prenatal (gestation and each trimester) and postnatal periods. Gestational exposure to PM_{10} increased the risks of infant mortality from all-cause and respiratory cause in normal birth weight infants per IQR change (6.93 $\mu g/m^3$). Gestational exposure to TSP and $PM_{2.5}$ also significantly elevated the risks of all-cause mortality in normal birth weight infants per IQR change (8.91 $\mu g/m^3$ and 3.15 $\mu g/m^3$, respectively). Gestational exposure to TSP, PM_{10}, and $PM_{2.5}$ increased the risk of respiratory-related mortality per IQR increase. When stratified by each trimester, these increases were only statistically significant for exposure during the first trimester.

10.2.2 Biological Mechanism

The biological mechanism by which particulate matter influences infant mortality has not been elucidated. It is plausible that particulate matter may induce oxidative stress, inflammation, and reduction of cell proliferation in different parts of the body [15–17] and thus adversely influence infant health.

10.2.3 Summary

Studies examining the association between particulate matter and infant mortality are few in number and are mostly concerned with short-term exposure. The present review suggests that particulate matter has an influence on infant mortality. However, more research is needed to enhance our understanding of the long-term effects of particulate matter on infant mortality. Furthermore, to advance our current understanding, refined analysis on the composition of particulate matter needs to be explored, as does the timing of exposure and how the specific components of particulate matter affect infant mortality. Even so, based on the current evidence, adequate public health interventions to prevent infants from particulate matter exposure can be delivered to the public (Table 10.2).

Table 10.2 Effects of particulate matter on infant mortality

Air pollution	Health outcome measurement (age)	Effect size	Reference (study design)
PM2.5 SPM PM7–2.5 Postnatal	Infant mortality (under 1-year-old)	Per 10 μg/m^3 increase in PM2.5 Infant mortality: AOR = 1.06(95% CI = 1.10–1.12) Post-neonatal mortality: AOR = 1.10 (95% CI = 1.02–1.19) Respiratory diseases: AOR = 1.30 (95% CI = 1.01–1.67) PM7 Post-neonatal mortality: AOR = 1.10 (95% CI = 1.03–1.16) Respiratory diseases: AOR = 1.25 (95% CI = 1.02–1.54) PM7–2.5 Post-neonatal mortality: AOR = 1.21 (95% CI = 1.03, 1.42)	Yorifuji et al. [12] (time-stratified, case-crossover design conditional logistic regression)
PM10 O3 Postnatal	Infant mortality (1- to 11-month-old infant)	Per 38.7 μg/m^3 (IQR) increase in PM10 Infant mortality at lag 1: AOR = 1.055 (95% CI = 1.011–1.102) Infant mortality at lag 2: AOR = 1.066 (95% CI = 1.021–1.114) Infant mortality at lag 0–2: AOR = 1.063 (95% CI = 1.001–1.132) Respiratory diseases lag 2: AOR = 1.098 (95% CI = 1.021–1.180) PM10 within O3 (quartiles first and fourth) At the highest O3 quartile (≥130.5 ppb) Infant mortality at lag 0: AOR = 1.145 (95% CI = 1.016–1.290) Infant mortality at lag 1: AOR = 1.139 (95% CI = 1.014–1.279) Infant mortality at lag 2: AOR = 1.131 (95% CI = 1.012–1.262) Infant mortality at lag 0–2: AOR = 1.261 (95% CI = 1.078–1.474)	Carbajal-Arroyo et al. [13] (time-stratified, case-crossover design conditional logistic regression)

TSP PM10 PM10–2.5 PM2.5 Prenatal Postnatal	Infant mortality Infant, omitted infants who died in the neonatal period (<28 days)	Per IQR increase in gestational exposure TSP (8.91 μg/m^3)—All (normal birth weight): AHR = 1.44 (95% CI = 1.06–1.97) PM10 (6.93 μg/m^3)—All (normal birth weight): AHR = 1.65 (95% CI = 1.18–2.31) PM2.5 (3.15 μg/m^3)—All (normal birth weight): AHR = 1.53 (95% CI = 1.22–1.90) First trimester (normal birth weight): AHR = 1.15 (95% CI = 1.04–1.28) Per IQR increase in gestational exposure Respiratory diseases (normal birth weight) TSP (8.91 μg/m^3)—All: AHR = 3.78 (95% CI = 1.18–12.13) First trimester: AHR = 2.08 (95% CI = 1.26–3.43) PM10 (6.93 μg/m^3)—All: AHR = 6.20 (95% CI = 1.50–25.66) First trimester: AHR = 2.19 (95% CI = 1.30–3.70) PM2.5 (3.15 μg/m^3)—All: AHR = 3.15 (95% CI = 1.26–7.85) First trimester: AHR = 1.58 (95% CI = 1.14–2.19)	Son et al. [14] (cohort Cox proportional hazards)

AOR adjusted odds ratio, *AHR* adjusted hazard ratio

10.3 Birth Outcomes

The role of the environment on the health of children has been widely researched, with findings suggesting an association between air pollution and various birth outcomes. Infants are most likely to be affected by the hazardous environment during the prenatal period.

The impacts of air pollution on birth outcomes are not trivial because air toxicants, among others, can be passed through the placenta from the mother to the fetus [18–20]. Therefore, during pregnancy, the environment that the fetus lives in and the toxicants that the fetus is exposed to are primarily dependent on the mother [18, 19]. Mothers' exposure to air pollution can be from within the home, known as household air pollution, or from outdoors, known as ambient air pollution [18, 19].

Moreover, birth outcomes are linked to morbidity, mortality, disability, and disease, with the consequences potentially being experienced through to adult life [18, 21]. Air pollution also has the potential to have societal impacts including an increased use of healthcare facilities after birth [18]. These examples highlight the importance of understanding the association between air pollution and birth outcomes as the consequences can be experienced by the individual and society as a whole, and this burden is entirely preventable.

Air pollution may influence common complications of pregnancy. Two of these complications are small for gestational age (SGA), which results from intrauterine growth retardation, and premature birth (PB). These conditions can occur independently or together, and both can result in low birth weight (LBW) [18].

10.3.1 Fetal Growth

Maternal exposure to air pollution can cause fetal growth stunting, which, in turn, is linked to the outcomes of LBW and SGA [22]. These birth outcomes have been associated with an increased risk of cardiovascular morbidity and mortality later in life and emerging evidence suggests an increased risk of developmental delays and lower intelligence [18, 23]. See the definitions below.

Def 3.1 Low Birth Weight (LBW) and Small for Gestational Age (SGA) As defined by WHO, low birth weight is a weight at birth of less than 2500 grams (5.5 lb) (http://apps.who.int/iris/bitstream/10665/43184/1/9280638327.pdf). LBW is a significant public health issue worldwide, and it is associated with a range of short- and long-term health effects. It is estimated that 15%–20% of all births worldwide are LBW, which represents more than 20 million births per year (http://www.who.int/nutrition/topics/globaltargets_lowbirthweight_policybrief.pdf).

In comparison, SGA refers to babies who are smaller than usual for the number of weeks of pregnancy, most commonly defined as having a birth weight below the 10th percentile of the recommended gender-specific birth weight for gestational age and gender [24]. Both LBW and SGA can be linked to preterm birth (PB) [18].

10.3.1.1 Low Birth Weight

Traffic-related air pollution is a major contributor to ambient air quality, and a study by Aguilera et al. investigated the effect of prenatal exposure to traffic-related air pollution on birth weight. The study used geographic information system (GIS) models on birth weight in 570 newborns from the INMA Sabadell cohort. A significant association between aromatic hydrocarbons (benzene, toluene, ethylbenzene, m/p-xylene, and o-xylene) and LBW was found, highlighting the negative impact traffic-related air pollutants can have on birth outcomes [3].

10.3.1.2 Low Birth Weight

Using spatiotemporal exposure metrics, a prospective cohort study, examining the relationship between LBW and exposure to various air pollutants (CO, NO, NO_2, $PM_{2.5}$, PM_{10}, O_3, and SO_2), found that residences within 50 m of highways were associated with an 11% increase in LBW (95% CI = 1.01–1.23). While ambient air pollution levels were detected to be relatively low, compared to air quality standards and international guidelines, the importance of reducing ambient air pollution and effective urban planning is evident [25].

While many studies on air pollution and LBW have looked at exposure throughout pregnancy, Darrow et al. [26] focused on exposure late in pregnancy. This five-country analysis, conducted between 1994 and 2004, found that ambient levels of NO_2, SO_2, $PM_{2.5}$, elemental carbon, $PM_{2.5}$ and water-soluble metals were all significantly associated with reductions in birth weight (−4 to −16 g per IQR increase in pollutant concentrations). Furthermore, this association was generally stronger in Hispanic and non-Hispanic black infants compared to non-Hispanic white infants, indicating a physiological difference between different ethnic groups.

Through a review of current literature, a number of additional studies show an association between air pollution and LBW (https://www.ncbi.nlm.nih.gov/pubmed/26918840, https://www.ncbi.nlm.nih.gov/pubmed/26137887, https://www.ncbi.nlm.nih.gov/pubmed/26046983).

10.3.1.3 Small for Gestational Age (SGA)

Gray et al. [27] assessed the association between $PM_{2.5}$ and O_3 exposure to individual- and area-based SES indicators and SGA from 2002 to 2006. Daily measurements of $PM_{2.5}$ and O_3 were calculated through a spatial hierarchical Bayesian

model. The researchers found that $PM_{2.5}$ air pollutants as well as maternal race, education, and neighborhood household income were associated with SGA (OR = 1.03, 95% CI = 1.02–1.05 per IQR). Despite the link between $PM_{2.5}$ air pollutants and SGA, O_3 showed inconsistent effects, substantiating the need for further research in this area. Woodruff et al. [28] reported disparities in air pollution exposure during pregnancy based on a multi-pollutant index by race, but not educational attainment in the USA. While in a study in Toronto, Buzzelli and Jerrett [29] found higher NO_2 exposure among both those with lower incomes and those with higher status occupations.

Stieb et al. [30] found that NO_2 was associated with adverse birth outcomes like SGA. Another study in Ohio showed that exposure to $PM_{2.5}$ in the last trimester was associated with an increased risk of SGA [31]. Ha et al. [32] found that elemental carbon exposure showed a 4% increase in SGA. Others studies have reported significant positive associations between NO_2 and LBW, SGA, or reduced birth weight [33, 34]. An association of a larger magnitude of $PM_{2.5}$ with SGA and term birth weight (but not with term LBW) was observed among births to mothers born in Canada [30].

Le et al. [35] investigated the link between SGA and exposure to SO_2, CO, NO_2, O_3, and PM_{10} during the first month and third trimester of pregnancy. The study found an association between SGA and CO levels that exceeded 0.75 ppm (OR = 1.14, 95% CI = 1.02–1.27) and NO_2 exceeding 6.8 ppb (OR = 1.11, 95% CI = 1.03–1.21) exposure in the first month, and PM_{10} exceeding 35 $\mu g/m^3$ (OR = 1.22, 95% CI = 1.03–1.46) and O_3 (OR = 1.11, 95% CI = 1.02–1.20) exposure in the third trimester.

Overall, there is growing evidence of the link between air pollution and the SGA birth outcome; however, many studies still present limited findings, thus highlighting the need for further research.

10.3.2 Preterm Birth (PB)

Maternal exposure to air pollutants during pregnancy has also been linked with preterm birth (PB), which is defined below.

Def 3.2 Preterm Birth (PB)
WHO defines PB as babies born alive before 37 weeks of gestation [5]. It is estimated that each year, 15 million babies are born preterm, with PB complications being the leading cause of death among children under 5 years of age (responsible for nearly one million deaths annually) (http://www.who.int/mediacentre/factsheets/fs363/en/).

As mentioned above, Le et al. [35] found a significant association between PB and SO_2 and O_3, but surprisingly not CO. This prospective cohort study included 164,905 singleton births among a large Black population in Detroit, Michigan between 1990 and 2001. Air pollutants were measured with three fixed site ambient air monitors located in densely populated urban areas. PB was associated with SO_2 (OR = 1.07, 95% CI = 1.01–1.14) exposure in the last month, with hourly O_3 exceeding 92 ppb (OR = 1.08, 95% CI = 1.02–1.14) exposure in the first month. This study also noted the importance of accounting for individual risk factors such as maternal smoking, maternal race, and long-term trends in air pollution levels and adverse birth outcomes. Additionally, the results showed that infants born to Black mothers had an approximately twofold higher risk of PB than those who were born to White mothers; however, further research is needed to support this finding.

Another study supporting the association between PB and air pollution [36] is a population-based case–control study in Los Angeles County, California. This study used three exposure data sources to examine the risks of PB among mothers exposed to high levels of traffic-related air pollutants during pregnancy. The odds of PB increased 6–21% per IQR increase in all pregnancy exposures to $PM_{2.5}$ as well as other traffic-related pollutants, while there was a 30% per interquartile increase in PAH (OR = 1.3, 95% CI = 1.15–1.47).

A retrospective cohort study in Brisbane, Australia supports the above findings, demonstrating an association between PB and air pollution [37]. This study assessed average maternal exposures to ambient PM_{10}, O_3, and NO_2 air pollutants of 28,200 singleton live births between 2000 and 2003, during the first 3 months after the last menstrual period and the last 3 months prior to birth. This study found that exposure to PM_{10} and O_3 in the first trimester was strongly associated with an increased risk of PB (OR = 1.15, 95% CI = 1.06–1.25 and OR = 1.26, 95% CI 1.10–1.45, respectively).

With the rate of PB across 184 countries ranging from 5% to 18% and evidence indicating an association with ambient air pollution, reducing exposure to ambient air pollution has the potential to drastically reduce the prevalence of PB (http://www.who.int/mediacentre/factsheets/fs363/en/).

10.3.3 Summary

Air pollution is strongly linked to LBW, PB, and SGA birth outcomes. As each of these birth outcomes has the potential to have a severe impact on the health of the child throughout his or her lifetime, it is critically important for all countries to ensure compliance with the WHO air quality guidelines (http://apps.who.int/iris/bitstream/10665/43184/1/9280638327.pdf). As air pollution has the potential to impact individual as well as societal health, this review highlights the importance of better understanding the association between air pollution and birth outcomes.

10.4 Neurocognitive Development

Neural development is a process that includes proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis of neurons [38]. Prenatal development is a critical window for the growth of the central nervous system (CNS) [39, 40]. Three recent systematic reviews have provided moderate evidence that air pollution affects children's neurodevelopment, which cannot be ignored [39, 41]. The evidence as a whole suggests that vehicular pollution contributes to cognitive impairment, thus emphasizing that government bodies and individuals should take prompt measures to control air pollution [42]. In this review, the effects of prenatal and postnatal air pollution exposure on children's neurodevelopment are considered separately. Only studies on ambient air pollution are included as well as studies on several cognitive functioning tests along with global intelligence quotients (IQ) and behavioral disorders, such as autism, ASD, and ADHD.

Def 4. Neurodevelopment, ASD, ADHD
Neurodevelopment is a process that includes the proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis of neurons [38]. Autism spectrum disorder (ASD) is a developmental disorder that affects communication and behavior. Although autism can be diagnosed at any age, it is said to be a "developmental disorder" because symptoms generally appear in the first 2 years of life (https://www.nimh.nih.gov/health/topics/autism-spectrum-disorders-asd/index.shtml). Attention-deficit/hyperactivity disorder (ADHD) is a brain disorder marked by an ongoing pattern of inattention and/or hyperactivity–impulsivity that interferes with functioning or development (https://www.nimh.nih.gov/health/topics/attention-deficit-hyperactivity-disorder-adhd/index.shtml).

10.4.1 Children's Neurocognitive Function–Prenatal Exposure

Lertxundi et al. [43] assessed children at the age of 15 months on the Bayley Scales of Infant Development (BSID) and concluded that prenatal $PM_{2.5}$ and NO_2 exposures were associated with significant decreases in neurocognitive scores.

Guxens et al. [40] suggested that exposure to fine particles during fetal life was related to structural alterations of the cerebral cortex in children's brains, and these alterations partially mediated the association between exposure to fine particles during fetal life and children's impaired inhibitory control.

A pooled analysis of six European prospective studies examining the association of prenatal exposure to $PM_{2.5}$, PM_{10}, and NO_2 with cognitive development [44] identified small deficits in global cognition, language development, and psychomotor

development with an increase in pollution exposure. However, only the association between increased NO_2 and a deficit in psychomotor development was significant. Among urban youth, Peterson et al. [45] conducted an MRI study exploring the effects of prenatal exposure to polycyclic aromatic hydrocarbons (PAHs) on the development of white brain matter, cognition, and behavior in later childhood. Results indicated that prenatal PAH air pollutant exposure is associated with cognitive and behavioral disturbances in childhood and disrupts the development of left-hemisphere white matter, which contributes to slower processing speed, ADHD symptoms, and externalizing problems in urban youth.

10.4.2 Children's Neurocognitive Function–Postnatal Exposure

In a Spanish, longitudinal study, Freire et al. [46] showed that high exposure to air pollution was associated with a modest decrease on the McCarthy Scales of Children's Abilities (MSCA) at 5 years of age. In another prospective birth cohort study, Suglia et al. [47] observed that an IQR increase in log-transformed black carbon exposure was associated with a reduction in scores of several subscales of the Kaufman Brief Intelligence Test (K-BIT) and the Wide Range Assessment of Memory and Learning (WRAML) at 9 years of age.

A Spanish longitudinal study [48] found that children exposed to higher levels of $PM_{2.5}$, black carbon, and ultrafine particles at school performed worse on tests of working memory and attentiveness than those exposed to lower levels. After 12 months, the gap widened significantly among those in the highly exposed group. Another longitudinal study [49] demonstrated a non-linear relationship between air pollution exposure and attention abilities. The second and third quartiles of black carbon exposure were associated with more errors and slower reaction times on the Connors' Continuous Performance Task. However, this relationship was heavily reduced among those in the highest quartile of black carbon exposure.

10.4.3 Prenatal and Postnatal Air Pollution Exposure and ASD and ADHD

Becerra et al. [50] reported an increased risk of ASD with increased NO_x, O_3, and $PM_{2.5}$ exposure. Similarly, three other studies assessed the association between pre- and postnatal exposure to $PM_{2.5}$ and ASD [51–53]. Another study in Spain [54] found that higher exposure to NO_2 during pregnancy is associated with impaired attentional function, especially increased inattentiveness, in children aged 4–5 years.

Postnatal exposure to NO_2 is also associated with increased inattentiveness, although it is difficult to completely dissociate the effects of pre- and postnatal

exposure since they are highly correlated. Jung et al. [55] reported increased odds of ASD in relation to postnatal exposure to CO, NO_2, O_3, and SO_2. Based on case–control studies, several systematic reviews on autism have described fairly consistent evidence showing an association between air pollution, especially prenatal exposure to PM, and an ASD diagnosis [56–58].

However, several European studies have not demonstrated any association between prenatal exposure to NO_2, $PM_{2.5}$, or PM_{10} and autistic traits [59] or between pre- and postnatal exposure to NO_x, PM_{10}, and ASD and ADHD [60]. A cross-sectional American study [61] also did not find any association between pre- and postnatal exposure to NO_x and PM_{10} and ADHD, respectively. Furthermore, a population-based nested case–control study in Israel [62] revealed that exposure to NO_2 during the postnatal period may be more relevant to ASD than prenatal exposure.

10.4.4 Biological Mechanism

The potential cellular mechanisms known to be responsible for CNS damage are neuroinflammation, oxidative stress, glial activation, and white matter injury [63, 64].

Further research is still needed on the specific components of air pollution that are responsible for CNS damage and the molecular mechanisms involved in humans [39].

10.4.5 Summary

Although current studies did determine the critical period of exposure (pre- or postnatal) for the occurrence of ASD [39], the evidence of an association between pre- or postnatal exposure to $PM_{2.5}$, PM_{10}, and ASD and ADHD is limited (Tables 10.3 and 10.4).

10.5 Childhood Obesity

Childhood obesity is emerging as a major public health problem that is on the increase worldwide [65]. The rates of overweight and obesity among youths have been shown to be highly prevalent in many countries, especially in the USA where the rate increased to about 32% in 2003–2006 [66, 67]. Childhood obesity can develop into serious diseases such as metabolic or cardiovascular disorders, and the rapid increase of its prevalence can be attributed to environmental factors in childhood [68, 69]. There has been growing interest in discovering the wider determinants of childhood obesity, but much more needs to be clarified, including the identification of distal and modifiable factors such as air pollution and traffic delays [65].

Table 10.3 Effects of air pollution on neurodevelopment

Air pollution	Health outcome measurement (age)	Effect size	Reference (study design)
NO_2 Prenatal	Psychomotor development (1–6 years)	−0.68 (−1.25 to −0.11)	Guxens et al. [44] Prospective studies
Aerosol samples measure $PM_{2.5}$, NO_2 Prenatal	Neurodevelopment *BSID* (15 months)	1 μg/m^3 increase in $PM_{2.5}$ in motor score (−0.14; −1.75, −0.53) 1 μg/m^3 increase in NO_2 in mental score (−0.29; −0.47, −0.11)	Yorifuji et al. [12] Longitudinal
SPM Prenatal	Survey questions about behavioral problems (8 years)	1.06 (95% CI: 1.01, 1.11) interrupting others, 1.09 (95% CI: 1.03, 1.15) failure to pay attention, 1.06 (95% CI: 1.01, 1.11) for lying, 1.07 (95% CI: 1.02, 1.13) for causing public disturbance	Suglia et al. [47] Prospective
Quartiles of average black carbon exposure estimated using LUR mean 0.56 μg/m^3 Postnatal	Cognitive functioning *K-BIT, WRAML* (8–11 year)	An interquartile range (0.4 μg/m^3) *Vocabulary* (−2.2, −5.5 to 1.1); *matrices* (−4.0, −7.6 to −0.5); *Composite* (−3.4, −6.6 to −0.3); *verbal* (−1.3, −4.8 to 2.2); *Visual* (−5.4, −8.9 to −1.9); *learning* (−2.8, −6.6 to 1.1); *General index* (−3.9,-7.5 to −0.3)	Freire et al. [46] Longitudinal
Home outdoor NO_2 concentration Estimated using LUR Postnatal 15.40 μg/m^3 15.40–24.75 μg/m^3 424.75 μg/m^3	Neurodevelopment *MSCA* (*Spanish*) (5 years) 0% female	Medium NO_2 exposure (β = −1.07, −9.99 to 7.85) High NO_2 exposure (β = −4.19, −14.02 to 5.64)	
Exposure to $PM_{2.5}$, black carbon and ultrafine particles at school *Ultrafine particles* 8034cm^3 (indoor) 11,939 cm^3 (outdoor)	Working memory *n-back attentional network* (7–10 years)	*Working memory (2-back):* Change from −5.3 (−1.6, 5.1) points to −9.9 (−16, −3.5) points *Working memory (3-back):* Change from −1.4 (−10, 7.1) points to −5.8 (−11, −0.74) points. *Inattentiveness:* Change from 5.2 (— 6.2, 17) points to 5.2 (0.68, 9.7) points (higher score = poorer performance).	Sunyer et al. [48] Longitudinal (Spain)

BSID-II Bayley scale of infant development – Revised, *CPT* Connors' continuous performance test, *DMST* digit memory span test, *DSST* digit symbol substitution test, *ETS* environmental tobacco smoke, *HECT* hand-eye coordination test, *IQ* intelligence quotient, *KBIT* Kaufman brief intelligence test, *MSCA* McCarthy scales of children's abilities, *NO_2* nitrogen dioxide, *OR* odds ratio; will be added

Table 10.4 Effects of air pollution on ASD and ADHD

Air pollution	Health outcome measurement (age)	Effect size	Reference (study design)
Per interquartile range (IQR) Particulate matter ≤2.5 μm Prenatal (entire pregnancy)	ASD DSM-IV-R (6–7 years)	OR = 1.15 (95% CI: 1.06–1.24) Per 4.68 μg/m^3 increase	Becerra et al. [50] Case–control
Per IQR increase $PM_{2.5}$ Prenatal and postnatal	ADOS (not provided)	*Postnatal* OR = 1.57 (95% CI: 1.22–2.03) *9 months of pregnancy* OR = 1.63 (95% CI: 1.08–2.47) *Third trimester* OR = 1.42 in PM2.5 (95% CI: 1.09–1.86)	Raz et al. [51] Nested case–control
Nitrogen dioxide, $PM_{2.5,}$ PM_{10} Prenatal, postnatal, and during the first year of life	ADI-R ADOS (2–5 years)	AOR, 1.81 (95% CI: 1.37–3.09) AOR, 2.08 (95% CI: 1.93–2.25) AOR, 2.17 (95% CI: 1.49–3.16) AOR, 2.06 (95% CI: 1.37–3.09) AOR, 2.12 (95% CI: 1.45–3.10) AOR, 2.14 (95% CI: 1.46–3.12).	Volk et al. [52] Case–control
$PM_{2.5}$ Prenatal and postnatal	ADOS SCQ (3–7 years)	*Postnatal year two* AOR = 1.45 (95% CI = 1.01–2.08) *Pre-pregnancy through year 2* OR = 1.51 (95% CI = 1.01–2.26)	Talbott et al. [53] Case–control
PM10: Per 10 μg/m^3 increase CO: Per 100 ppb in CO NO_2, O_3: Per 10 ppb Increase postnatal SO_2: Per 1 ppb in SO_2 Multi-pollutant models	ICD-9-CM 6.26 (2.91 years)	59% risk increase l (95% CI 1.42–1.79), 37% risk increase (95% CI 1.31–1.44), 340% risk increase (95% CI 3.31–5.85), 17% risk increase level (95% CI 1.09–1.27)	Jung et al. [55] Prospective cohort (I)
NO_x $PM_{2.5}$ PM_{10} Prenatal	Autistic traits A-TAC (4–10 years)	Borderline/clinical range *Per each 10 μg/m^3 increase in NO_2* OR = 0.94 (95% CI: 0.81–1.10) *PM2.5, PM10*: NS	Guxens et al. [59] Birth cohorts and child cohort
NO_x PM_{10} Prenatal	ASD, ADHD A-TAC (9–12 years)	NO_x 5–95% difference, odds ratios (ORs) of 0.92 (95% CI: 0.44–1.96) for ASD 0.90 (95% CI: 0.58–1.40) for ADHD *PM10* ORs of 1.01 (95% CI: 0.52–1.96) for ASD 1.00 (95% CI: 0.68–1.47) for ADHD	Gong et al. [60] Birth cohort (I)

Although only a few epidemiological studies have investigated the association of air pollution with childhood obesity, two recent systematic reviews concluded that outdoor air pollution significantly contributes to the development of obesity in childhood [70, 71]. However, additional evidence is required indicating an association between air pollution and childhood obesity. According to one systematic review, the evidence on this issue is "insufficient" because of the small number of studies [72].

This review includes studies of ambient air pollution only and childhood obesity, which is defined by weight-for-length or body mass index (BMI) and metabolic syndrome.

Def 5. Childhood Obesity
The body mass index (BMI) is a measure used to determine childhood overweight and obesity. Overweight is defined as a BMI at or above the 85th percentile and below the 95th percentile for children and teens of the same age and sex. Obesity is defined as a BMI at or above the 95th percentile for children and teens of the same age and sex. The BMI is calculated by dividing a person's weight in kilograms by the square of height in meters. For children and teens, the BMI is age- and sex-specific and is often referred to as BMI-for-age. A child's weight status is determined using an age- and sex-specific percentile for the BMI rather than the BMI categories used for adults. This is because a child's body composition varies as he or she ages, and it varies between boys and girls. Therefore, the BMI levels among children and teens must be expressed relative to other children and teens of the same age and sex (https://www.cdc.gov/obesity/childhood/defining.html).

10.5.1 Air Pollution Effects on Childhood Obesity

Fleisch et al. [73] assessed the weights and lengths of US infants at birth and 6 months of age and examined the association of prenatal $PM_{2.5}$ and black carbon with fetal growth and infant weight gain among participants of the project Viva cohort. Results showed lower fetal growth among infants exposed to the highest quartile of black carbon during the third trimester compared to the lowest quartile group. In addition, although effect estimates were imprecise, the highest quartile of black carbon or $PM_{2.5}$ exposure during the third trimester was also shown to be positively associated with 0–6 months weight-for-length gain. Furthermore, compared with the lowest quartile of neighborhood traffic density, infants of the highest quartile had a greater weight-for-length gain from 0–6 months of age, and they had greater odds of weight-for-length $\geq$ the 95th percentile at 6 months of age.

Kim et al. [74] indicated that higher exposure to early life near-roadway air pollution (NRAP) increased the rate of change of childhood BMI and resulted in a higher attained BMI at 10 years of age that was independent of later childhood

exposures. These findings suggest that elevated early life NRAP exposure contributes to an increased risk of obesity in children. Moreover, results indicated that increased first year of life near-road freeway NO_x exposures are associated with an increased velocity of childhood BMI growth trajectory and higher attained BMI at 10 years. Furthermore, increased childhood near-roadway exposures from non-freeway sources were associated with increased BMI growth and a higher BMI at 10 years.

In another longitudinal study in the USA, Rundle et al. [75] suggested that prenatal exposure to the PAH of ambient traffic-related pollution was associated with an increased BMI and obesity at 5 and 7 years of age. When the prenatal PAH exposure levels were divided into three groups based on the concentrations of exposure, children of mothers in the highest exposure group had a higher BMI z-score and a relative risk of 1.79 for obesity at 5 years of age. They also had a higher BMI z-score, a higher percentage of body fat, and a relative risk of 2.26 for obesity at 7 years of age, compared with children of mothers in the lowest group of PAH exposure.

Huang et al. [76] assessed the association of air pollutants (PM_{10}, SO_2, NO, and NO_2) at different growth phases (in utero, in infancy, and in childhood) with a BMI at ~9, ~11, ~13, and ~15 years of age in a population-representative birth cohort from Hong Kong, "Children of 1997." This study found that associations were sex-specific based on better model fit when including sex interaction terms. Among boys, higher NO_2 in childhood was associated with higher BMI at ~9, ~13, and ~15 years of age using a multi-pollutant model.

The GINIplus and LISAplus birth cohorts study [77] showed insulin resistance increased by 17.0% (95% CI 5.0, 30.3) and 18.7% (95% CI 2.9, 36.9) for every 2-SD increase in ambient NO_2 and particulate matter $\leq$10 μm in diameter, respectively, indicating that traffic-related air pollution may increase the risk of insulin resistance. Given the ubiquitous nature of air pollution and the high incidence of insulin resistance in the general population, the associations examined here may have potentially important public health effects despite the small/moderate effect sizes observed.

10.5.2 Biological Mechanism

It is plausible that air pollutants are potent oxidizers that act either directly on lipids and proteins or indirectly through the activation of intracellular oxidant pathways [78, 79]. Oxidative stress caused by exposure to air pollutants may therefore play a major role in the development of insulin resistance [77, 80]. Another hypothesis is one centered on environmental obesogens, which are chemical simulators of metabolic hormones or brain neurotransmitters [81, 82]. The hypothesis builds on the existing science that chemicals in air pollution have the potential to interfere with

endocrine and metabolic systems and may change growth patterns and induce weight gain, obesity, and obesity-related diseases such as metabolic syndromes and cardiovascular diseases (CVD) [72, 74, 82–84].

10.5.3 Summary

Epidemiological studies examining the associations between exposure to air pollution and childhood obesity with insulin resistance are scarce. The present review may suggest a positive association between outdoor air pollution, especially PM and NO_2, and the development of obesity or insulin resistance in children. These air pollutants may disrupt normal development and thus result in increased weight-for-length gain, mean BMI growth, and differences in attained BMI at specific ages [74–76]. Many research questions remain, and further studies are needed to fill the data gaps to stimulate focused research and advance the field. Taking into account the current knowledge on the adverse effects of obesogen chemicals on childhood health, the child obesity epidemic should be considered a multifactorial complex disorder necessitating an emphasis on public health interventions for environmental protection [82] (Table 10.5).

Table 10.5 Effects of air pollution on child obesity

Air pollution	Health outcome measurement (age)	Effect size	Reference (study design)
$PM_{2.5}$ and black carbon Prenatal	Fetal growth and infant weight gain (6 months)	The highest (vs. lowest) quartile of neighborhood traffic density: Increased weight-for-length gain z-score change, (β) = 0.25 (0.01 to 0.49) Greater odds of weight-for-length ≥95th percentile at 6 months (OR) = 1.84 (1.11 to 3.05)	Fleisch et al. [73] Cohort (USA)
Polycyclic aromatic hydrocarbon (PAH) Prenatal	BMI (body mass index) (age 5 and 7 years)	The highest (vs. lowest) prenatal PAH: Higher BMI z-score At 5 years (β) = 0.39 (0.08–0.70) At 7 years (β) =0.30 (0.01–0.59) risks of obesity At 5 years (RR) = 1.79 (1.08–2.98) At 7 years (RR) = 2.26 (1.28–4.00)	Rundle et al. [75] Cohort (USA)
Ambient NO_2 and particulate matter ≤10 μm At birth	HOMA of insulin resistance (HOMA-IR) (10-year-old children)	Insulin resistance increased by 17.0% (95% CI 5.0, 30.3) and 18.7% (95% CI 2.9, 36.9) for every 2SDs increase in ambient NO_2 and particulate matter ≤10 μm in diameter	Thiering et al. [77] Two birth cohorts (Germany)

References

1. WHO. Ambient (outdoor) air quality and heath. Fact sheet. 2016. http://www.who.int/mediacentre/factsheets/fs313/en/. Accessed 2 May 2018.
2. World Health Organization. Don't pollute my future! The impact of the environment on children's health. Geneva: WHO; 2017.
3. Schwartz J. Air pollution and children's health. Pediatrics. 2004;113(3):1037–43.
4. Dominici F, Peng RD, Bell ML, Pham L, McDermott A, Zeger SL, Samet JM. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA. 2006;295(10):1127–34.
5. Schwartz J, Slater D, Larson TV, Pierson WE, Koenig JO. Particulate air pollution and hospital emergency room. Am Rev Respir Dis. 1993;147:826–31.
6. Dockery DW, Arden Pope C. Acute respiratory effects of particulate air pollution. Annu Rev Public Health. 1994;15(1):107–32.
7. Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, Luepker R, et al. Air pollution and cardiovascular disease. Circulation. 2004;109(21):2655–71.
8. Ha E-H, Lee J-T, Kim H, Hong Y-C, Lee B-E, Park H-S, Christiani DC. Infant susceptibility of mortality to air pollution in Seoul, South Korea. Pediatrics. 2003;111(2):284–90.
9. Šrám RJ, Binková B, Dejmek J, Bobak M. Ambient air pollution and pregnancy outcomes: a review of the literature. Environ Health Perspect. 2005;113:375–82.
10. Glinianaia SV, Rankin J, Bell R, Pless-Mulloli T, Howel D. Does particulate air pollution contribute to infant death? A systematic review. Environ Health Perspect. 2004;112:1365–70.
11. Jung EM. PhD paper, Ewha Womans University; 2018.
12. Yorifuji T, Kashima S, Doi H. Acute exposure to fine and coarse particulate matter and infant mortality in Tokyo, Japan (2002–2013). Sci Total Environ. 2016;551:66–72.
13. Carbajal-Arroyo L, Miranda-Soberanis V, Medina-Ramón M, Rojas-Bracho L, Tzintzun G, Solís-Gutiérrez P, Méndez-Ramírez I, Hurtado-Díaz M, Schwartz J, Romieu I. Effect of PM10 and O3 on infant mortality among residents in the Mexico City metropolitan area: a case-crossover analysis, 1997–2005. J Epidemiol Community Health. 2010;65:715–21.
14. Son J-Y, Bell ML, Lee J-T. Survival analysis of long-term exposure to different sizes of airborne particulate matter and risk of infant mortality using a birth cohort in Seoul, Korea. Environ Health Perspect. 2011;119(5):725.
15. Pinkerton KE, Zhou Y, Zhong C, Smith KR, Teague SV, Kennedy IM, Ménache MG. Mechanisms of particulate matter toxicity in neonatal and young adult rat lungs. Res Rep Health Eff Inst. 2008;135:3–41.
16. Kannan S, Misra DP, Timothy Dvonch J, Krishnakumar A. Exposures to airborne particulate matter and adverse perinatal outcomes: a biologically plausible mechanistic framework for exploring potential. Cien Saude Colet. 2007;12(6):1591–602.
17. Maier KL, Alessandrini F, Beck-Speier I, Hofer TPJ, Diabaté S, Bitterle E, Stöger T, et al. Health effects of ambient particulate matter—biological mechanisms and inflammatory responses to in vitro and in vivo particle exposures. Inhal Toxicol. 2008;20(3):319–37.
18. Landrigan PJ, Etzel RA. Textbook of children's environmental health. New York, NY: Oxford University Press; 2014.
19. Etzel RA, Balk SJ. American academy of pediatrics committee on environmental health. In: Pediatric environmental health. 2nd ed. Elk Grove Village, IL: American Academy of Pediatrics; 2002.
20. Baïz N, Slama R, Béné MC, Charles MA, Kolopp-Sarda MN, Magnan A, Thiebaugeorges O, Faure G, Annesi-Maesano I. Maternal exposure to air pollution before and during pregnancy related to changes in newborn's cord blood lymphocyte subpopulations. The EDEN study cohort. BMC Pregnancy Childbirth. 2011;11:87. https://doi.org/10.1186/1471-2393-11-87.
21. Zheng T, et al. Effects of environmental exposures on fetal and childhood growth trajectories. Ann Glob Health. 2016;82(1):41–99.
22. Vrijheid M, Casas M, Gason M, Valvi D, Nieuwenhuijsen M. Environmental pollutants and child health—a review of recent concerns. Int J Hyg Environ Health. 2016;219:331–42.

23. Lundgren EM, Tuvemo T. Effects of being born small for gestational age on long-term intellectual performance. Best Pract Res Clin Endocrinol Metab. 2008;22(3):447–88.
24. WHO. Expert committee report: physical status: the use and interpretation of anthropometry. Technical report series. Geneva: World Health Organization; 1995. p. 854.
25. Brauer M, Lencar C, Tarnburic L, Koehoorn M, Demers P, Karr C. A cohort study of traffic-related air pollution impacts on birth outcomes. Environ Health Perspect. 2008;116:680–6.
26. Darrow LA, Klein M, Strickland MJ, Mulholland JA, Tolbert PE. Ambient air pollution and birth weight in full-term infants in Atlanta, 1994-2004. Environ Health Perspect. 2011;119:731–7.
27. Gray SC, Edwards SE, Schultz BD, Miranda ML. Assessing the impact of race, social factors and air pollution on birth outcomes: a population-based study. Environ Health. 2014;13:4.
28. Woodruff TJ, Parker JD, Kyle AD, Schoendorf KC. Disparities in exposure to air pollution during pregnancy. Environ Health Perspect. 2003;111:942–6.
29. Buzzelli M, Jerrett M. Geographies of susceptibility and exposure in the city: environmental inequity of traffic-related air pollution in Toronto. Can J Reg Sci. 2007;30:195–210.
30. Stieb DM, Chen L, Hystad P, Beckerman BS, Jerrett M, Tjepkema M, Crouse DL, Omariba DW, Peters PA, van Donkelaar A, Martin RV. A national study of the association between traffic-related air pollution and adverse pregnancy outcomes in Canada, 1999–2008. Environ Res. 2016;148:513–26.
31. Percy Z, DeFranco E, Xu F, Hall ES, Haynes EN, Jones D, Muglia LJ, Chen A. Trimester specific PM2. 5 exposure and fetal growth in Ohio, 2007–2010. Environ Res. 2019;171:111–8.
32. Ha S, Zhu Y, Liu D, Sherman S, Mendola P. Ambient temperature and air quality in relation to small for gestational age and term low birthweight. Environ Res. 2017;155:394–400.
33. Ballester F, Estarlich M, Iniguez C, Llop S, Ramon R, Esplugues A, Lacasana M, Rebagliato M. Air pollution exposure during pregnancy and reduced birth size: a prospective birth cohort study in Valencia. Spain Environ Health. 2010;9:6.
34. Malmqvist E, Rignell-Hydbom A, Tinnerberg H, Björk J, Stroh E, Jakobsson K, Rittner R, Rylander L. Maternal exposure to air pollution and birth outcomes. Environ Health Perspect. 2011;119:553–8.
35. Le HQ, Battterman SA, Wirth JJ, et al. Air pollutant exposure and preterm and term small-for-gestational-age births in Detroit, Michigan: long-term trends and associations. Environ Int. 2012;44:7–17.
36. Wilhelm M, Ghosh JK, Su J, Cockburn M, Jerrett M, Ritz B. Traffic-related air toxics and preterm birth: a population-based case-control study in los Angeles County, California. Environ Health. 2011;10:89.
37. Hansen C, Neller A, Williams G, Simpson R. Maternal exposure to low levels of ambient air pollution and preterm birth in Brisbane, Australia. Int J Obstet Gynaecol. 2006;113(8):935–41.
38. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect. 2000;108(Suppl 3):511–33.
39. Suades-González E, Gascon M, Guxens M, Sunyer J. Air pollution and neuropsychological development a review of the latest evidence. Endocrinology. 2015;156(10):3473–82.
40. Guxens M, Lubczyńska MJ, Muetzel RL, Dalmau-Bueno A, Jaddoe VW, Hoek G, van der Lugt A, Verhulst FC, White T, Brunekreef B, Tiemeier H. Air pollution exposure during fetal life, brain morphology, and cognitive function in school-age children. Biol Psychiatry. 2018;84:295–303.
41. Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. Environmental pollutants and child health—a review of recent concerns. Int J Hyg Environ Health. 2016;219(4–5):331–42. https://doi.org/10.1016/j.ijheh.2016.05.001.
42. Clifford A, Lang L, Chen R, Anstey KJ, Seaton A. Exposure to air pollution and cognitive functioning across the life course—a systematic literature review. Environ Res. 2016;147:383–98. https://doi.org/10.1016/j.envres.2016.01.018.
43. Lertxundi A, Baccini M, Lertxundi N, Fano E, Aranbarri A, Martínez MD, Ayerdi M, Álvarez J, Santa-Marina L, Dorronsoro M, Ibarluzea J. Exposure to fine particle matter, nitrogen diox-

ide and benzene during pregnancy and cognitive and psychomotor developments in children at 15 months of age. Environ Int. 2015;80:33–40.
44. Guxens M, Garcia-Esteban R, Giorgis-Allemand L, Forns J, Badaloni C, Ballester F, Beelen R, Cesaroni G, Chatzi L, de Agostini M, de Nazelle A. Air pollution during pregnancy and childhood cognitive and psychomotor development: six European birth cohorts. Epidemiology. 2014;25(5):636–47.
45. Peterson BS, Rauh VA, Bansal R, et al. Effects of prenatal exposure to air pollutants (polycyclic aromatic hydrocarbons) on the development of brain White matter, cognition and behavior in later childhood. JAMA Psychiat. 2015;72(6):531–40. https://doi.org/10.1001/jamapsychiatry.2015.57.
46. Freire C, Ramos R, Puertas R, Lopez-Espinosa M, Julvez J, Aguilera I, Cruz F, Fernandez MF, Sunyer J, Olea N. Association of traffic-related air pollution with cognitive development in children. J Epidemiol Community Health. 2010;64:223–8.
47. Suglia SF, Gryparis A, Wright RO, Schwartz J, Wright RJ. Association of black carbon with cognition among children in a prospective birth cohort study. Am J Epidemiol. 2008;167:280–6.
48. Sunyer J, Esnaola M, Alvarez-Pedrerol M, Forns J, Rivas I, López-Vicente M, Suades-González E, Foraster M, Garcia-Esteban R, Basagaña X, Viana M, Cirach M, Moreno T, Alastuey A, Sebastian-Galles N, Nieuwenhuijsen M, Querol X. Association between traffic-related air pollution in schools and cognitive development in primary school children: a prospective cohort study. PLoS Med. 2015;12:e1001792.
49. Chiu YH, Bellinger DC, Coull BA, Anderson S, Barber R, Wright RO, Wright RJ. Associations between traffic-related black carbon exposure and attention in a prospective birth cohort of urban children. Environ Health Perspect. 2013;121:859–64.
50. Becerra TA, Wilhelm M, Olsen J, Cockburn M, Ritz B. Ambient air pollution and autism in Los Angeles county, California. Environ Health Perspect. 2013;121(3):380–6.
51. Raz R, Roberts AL, Lyall K, et al. Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case-control analysis within the Nurses' health study II cohort. Environ Health Perspect. 2015;123(3):264–70.
52. Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R. Traffic-related air pollution, particulate matter and autism. JAMA Psychiat. 2013;70(1):71–7.
53. Talbott EO, Arena VC, Rager JR, et al. Fine particulate matter and the risk of autism spectrum disorder. Environ Res. 2015;140:414–20.
54. Sentís A, Sunyer J, Dalmau-Bueno A, Andiarena A, Ballester F, Cirach M, Estarlich M, Fernández-Somoano A, Ibarluzea J, Íñiguez C, Lertxundi A. Prenatal and postnatal exposure to NO_2 and child attentional function at 4–5 years of age. Environ Int. 2017;106:170–7.
55. Jung C-R, Lin Y-T, Hwang B-F. Air pollution and newly diagnostic autism spectrum disorders: a population-based cohort study in Taiwan. PLoS One. 2013;8(9):e75510.
56. Lyall K, Schmidt RJ, Hertz-Picciotto I. Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol. 2014;43(2):443–64.
57. Rossignol DA, Genuis SJ, Frye RE. Environmental toxicants and autism spectrum disorders: a systematic review. Transl Psychiatry. 2014;4:e360.
58. Ornoy A, Weinstein-Fudim L, Ergaz Z. Prenatal factors associated with autism spectrum disorder (ASD). Reprod Toxicol. 2015;56:155–69.
59. Guxens M, Ghassabian A, Gong T, et al. Air pollution exposure during pregnancy and childhood autistic traits in four European population-based cohort studies: the ESCAPE project. Environ Health Perspect. 2015;124:133–40. https://doi.org/10.1289/ehp.1408483.
60. Gong T, Almqvist C, Bölte S, et al. Exposure to air pollution from traffic and neurodevelopmental disorders in Swedish twins. Twin Res Hum Genet. 2014;17(6):553–62.
61. Abid Z, Roy A, Herbstman JB, Ettinger AS. Urinary polycyclic aromatic hydrocarbon metabolites and attention/deficit hyperactivity disorder, learning disability, and special education in U.S. children aged 6 to 15. J Environ Public Health. 2014;2014:628508.
62. Raz R, Levine H, Pinto O, Broday DM, Yuval, Weisskopf MG. Traffic-related air pollution and autism Spectrum disorder: a population-based nested case-control study in Israel. Am J Epidemiol. 2018;187(4):717–25.

63. Mills NL, Donaldson K, Hadoke PW, et al. Adverse cardiovascular effects of air pollution. Nat Clin Pract Cardiovasc Med. 2009;6(1):36–44.
64. Block ML, Calderón-Garcidueñas L. Air pollution: mechanisms of neuroinflammation and CNS disease. Trends Neurosci. 2009;32(9):506–16.
65. Maziak W, Ward K, Stockton M. Childhood obesity: are we missing the big picture? Obes Rev. 2008;9(1):35–42.
66. Ogden CL, Carroll MD, Flegal KM. High body mass index for age among US children and adolescents, 2003–2006. J Am Med Assoc. 2008;299(20):2401–5.
67. Popkin BM. Recent dynamics suggest selected countries catching up to US obesity. Am J Clin Nutr. 2010;91(1):284s–8s.
68. Hill JO, Peters JC. Environmental contributions to the obesity epidemic. Science. 1998;280(5368):1371–4.
69. Dong GH, Qian Z, Liu MM, Wang D, Ren WH, Fu Q, et al. Obesity enhanced respiratory health effects of ambient air pollution in Chinese children: the seven northeastern cities study. Int J Obesity. 2013;37(1):94–100.
70. Kelishadi R. Poursafa P. A review on the genetic, environmental, and lifestyle aspects of the early-life origins of cardiovascular disease. Curr Probl Pediatr Adolesc Health Care. 2014;44(3):54–72.
71. Backes CH, Nelin T, Gorr MW, Wold LE. Early life exposure to air pollution: how bad is it? Toxicol Lett. 2013;216(1):47–53.
72. Vrijheid M, Casas M, Gascon M, Valvi D, Nieuwenhuijsen M. Environmental pollutants and child health—a review of recent concerns. Int J Hyg Environ Health. 2016;219(4–5):331–42.
73. Fleisch AF, Rifas-Shiman SL, Koutrakis P, Schwartz JD, Kloog I, Melly S, et al. Prenatal exposure to traffic pollution: associations with reduced fetal growth and rapid infant weight gain. Epidemiology. 2015;26(1):43–50.
74. Kim JS, Alderete TL, Chen Z, Lurmann F, Rappaport E, Habre R, et al. Longitudinal associations of in utero and early life near-roadway air pollution with trajectories of childhood body mass index. Environ Health. 2018;17(1):64.
75. Rundle A, Hoepner L, Hassoun A, Oberfield S, Freyer G, Holmes D, et al. Association of childhood obesity with maternal exposure to ambient air polycyclic aromatic hydrocarbons during pregnancy. Am J Epidemiol. 2012;175(11):1163–72.
76. Huang JV, Leung GM, Schooling CM. The association of air pollution with body mass index: evidence from Hong Kong's "children of 1997" birth cohort. Int J Obes. 2019;43(1):62–72.
77. Thiering E, Cyrys J, Kratzsch J, Meisinger C, Hoffmann B, Berdel D, et al. Long-term exposure to traffic-related air pollution and insulin resistance in children: results from the GINIplus and LISAplus birth cohorts. Diabetologia. 2013;56(8):1696–704.
78. Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. J Toxicol. 2011;2011:487074.
79. Moller P, Loft S. Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. Environ Health Perspect. 2010;118(8):1126–36.
80. Brook RD, Sun Z, Brook JR, Zhao X, Ruan Y, Yan J, et al. Extreme air pollution conditions adversely affect blood pressure and insulin resistance: the air pollution and Cardiometabolic disease study. Hypertension. 2016;67(1):77–85.
81. Grun F, Blumberg B. Minireview: the case for Obesogens. Mol Endocrinol. 2009;23(8):1127–34.
82. Kelishadi R, Poursafa P, Jamshidi F. Role of environmental chemicals in obesity: a systematic review on the current evidence. J Environ Public Health. 2013;2013:896789.
83. Thayer KA, Heindel JJ, Bucher JR, Gallo MA. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. Environ Health Perspect. 2012;120(6):779–89.
84. McConnell R, Gilliland FD, Goran M, Allayee H, Hricko A, Mittelman S. Does near-roadway air pollution contribute to childhood obesity? Pediatr Obes. 2016;11(1):1–3.

Chapter 11
Mercury, Lead, Manganese, and Hazardous Metals

Ching-Chung Lin, Meng-Shan Tsai, Mei-Huei Chen, and Pau-Chung Chen

Abstract Heavy metals are widely distributed as variety of forms in nature and the human living environment. Their health effect derived from diverse and unique chemical properties and biological actions is a traditional environmental issue, but yet important up to date.

Mercury and lead are well-known neurotoxins. Their exposure levels among the vulnerable fetus and children declined along with The Minamata Convention, regulation of lead-based paint and leaded gasoline. Adverse health impacts are still reported with low-level exposure. Manganese (Mn) is another neurotoxic metal, but only when exposed at high levels. The necessity for maintaining normal cell function makes it more complicated to explore the health impact of Mn exposure. Additionally, there is a growing attention on some heavy metals derived from industrial processes, such as vanadium (V) and thallium (Tl). Therefore, this current review will focus on recent reports of the aforementioned heavy metals toxicity

C.-C. Lin · M.-S. Tsai
Institute of Environmental and Occupational Health Sciences, National Taiwan University College of Public Health, Taipei, Taiwan

M.-H. Chen
Institute of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan

Department of Pediatrics, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

P.-C. Chen (✉)
Institute of Environmental and Occupational Health Sciences, National Taiwan University College of Public Health, Taipei, Taiwan

Department of Public Health, National Taiwan University College of Public Health, Taipei, Taiwan

Department of Environmental and Occupational Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan

Office of Occupational Safety and Health, National Taiwan University College of Medicine and Hospital, Taipei, Taiwan

Innovation and Policy Center for Population Health and Sustainable Environment, National Taiwan University College of Public Health, Taipei, Taiwan
e-mail: pchen@ntu.edu.tw

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_11

based on birth cohort studies with a particular consideration on the susceptible periods of pregnancy and early infancy. Some of the newly developed investigation tools such as epigenetic are also included.

Keywords Mercury · Lead · Manganese · Vanadium · Thallium · Pregnancy outcome · Neurodevelopment · Birth cohorts

11.1 Mercury (Hg)

Mercury (Hg) occurs in three different forms and varies in degree of toxicity. Elemental mercury is a liquid at room temperature and readily volatilizes to vapor which may cause significant damage to central nervous system (CNS) and renal system via inhalation. Nevertheless, absorption from ingestion is usually poor for elemental mercury and inorganic mercury except for mercuric salts. Ingestion of mercuric bichloride (Hg2+) can produce gastrointestinal tract ulceration or perforation rapidly as well as acute renal toxicity. The toxicity of organic mercury occurs with long-term exposure and affects the CNS. Minamata disease, first discovered in the 1950s, is a neurological syndrome caused by high methylmercury (MeHg) exposure through human consumption of fish contaminated by industrial release of mercury. Organic mercury is also a potent teratogen that could pass through the placenta and transfer into human milk. Some women who were pregnant during the Minamata Bay disaster and were asymptomatic or had exhibited mild toxic effects due to the Hg exposure gave birth to severely affected infants such as psychomotor retardation, blindness, deafness, or seizure developed over time.

Global action had been taken to reduce mercury pollution. The Minamata Convention on mercury was formally adopted in 2013 and entered into force in 2017 with the aim to protect human health and the environment from anthropogenic emissions and releases of mercury and mercury compounds. The treaty sets a phase-out date of 2020 for a list of products and to end all mercury mining within 15 years. Mercury thermometers and vaccines containing thimerosal as preservatives are all on the list. In addition, some EU (European Union) countries, Canada, and Japan have banned or restricted the use of dental amalgam (contains 40% to 50% mercury) in young children and women during pregnancy.

Organic Hg can be converted from environmental mercury in water and sediment by bacteria and is accumulated in the food chain. Therefore, regulation of Hg in drinking water or recommendations on fish consumption should be provided to avoid excess methylmercury exposure especially for susceptible populations. Shark,

king mackerel, tilefish, and swordfish were classified as high levels of mercury concentration by the United States Environmental Protection Agency (US EPA). The US EPA suggested a reference dose for Hg should be established at 0.1μg/kg per day to achieve a cord blood concentration of <5.8μg mercury/L—a concentration below which no adverse neurobehavioral toxicity is expected [1]. Review of the quantified contribution to total mercury in the US seafood revealed that most fish with the highest mercury levels are relatively minor contributors to total inputs except for swordfish [2]. Therefore, regional surveys, recommendations, and risk communication are warranted.

During the past half century, there have been significant declines in total mercury levels in whole blood, cord blood, and breast milk worldwide, and the trends are more obvious in Europe and Asia. By continent, the order of Hg levels from high to low is South America, Africa, Asia, and Europe or North America. Through model estimation, Sharma et al. [3] point out the concern of high blood Hg levels in children in Africa and contribution to cord blood by diet mercury [3]. Also, fish consumption was reported as a main predictor for blood Hg of mid/late-term pregnant women in Japan as well as cord blood mercury level in an inland country [4, 5].

The gastrointestinal tract absorbs about 95% of ingested MeHg derived from aquatic food. MeHg can establish specific chemical bonds with tissue proteins in the bloodstream and is excreted in feces via transportation out of liver cell into bile. Although the uptake of MeHg into brain is slow, the brain system has strong affinity for it. MeHg has a long half-life of around 70–80 days in the human body and is capable of crossing the placental barrier and accumulates in placental tissues. Therefore, the threat from MeHg continues to exist.

Recently, scientific research focuses on chronic exposure to low levels of Hg and adverse health conditions among vulnerable population such as children or pregnant women. One of the major concerns is neurotoxicity by interfering with normal cellular function and detoxification process. Data from three longitudinal studies of subsistence fishing populations concluded a dose–response relationship for child loss 0.18 of intelligence quotient (IQ) points by each increase of 1 part per million (ppm) in maternal hair mercury [6]. In a similar estimation by the World Health Organization (WHO), 1.5 to 17 out of 1000 children from selected subsistence fishing population show cognitive impacts caused by consumption of fish containing mercury [7]. Due to the variability of individual response to detrimental mercury action, the role of genetic polymorphisms had been explored by both toxicokinetics and epidemiological studies [8, 9]. However, the health impacts on immune system, metabolic system, or liver/renal function were less conclusive [3]. In order to further understand the influence of environmental mercury exposure during prenatal or early postnatal period, our current review focuses on recent reports from cohort studies, including fetal growth and pregnancy outcomes, and neurodevelopment and behavioral problems (Table 11.1). The details are described below.

Table 11.1 Association between mercury (Hg) and children's health outcomes based on cohort study

Location, reference	Year	Sample size	Specimen (I-infant; C-cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (–no or weak association; +association)
Fetal growth and pregnancy outcome					
China [10]	2011.6–2011.8	81	C, M	Birth weight	–
China [11]	2011	50	C, M, H, placenta	Neonates (weight and height) and infants (height)	+ (negative association)
Canada [12]	2008–2011	1835	M	Small for gestational age	+ (negative association)
China [13]	2011.7–2012.5	103	C	Birth weight, length, and head circumference	+ (negative association)
Spain [14]	2004–2008	1869	C	Birth anthropometry (weight, length, and head circumference), placental weight and gestational length	+ (negative association)
Korea, Taiwan [15]	2006–2010 (Korea) 2004–2005 (Taiwan)	895 (Korea) 252 (Taiwan)	C, M	Birth weight	+ (negative association)
Faroe Islands [16]	1997–2000	604	C, H	Gestational diabetes mellitus, birth size	–
Japan [17]	2003	489	C	Birth weight	+ (negative association)
Japan [18]	2010.12–2012.10	334 mother–child pair	M	Birth weight	+ (negative association)
Spain [19]	2003	1867	C	Fetal growth (Biparietal diameter, femur length, abdominal circumference, estimated fetal weight)	+ (negative association for BPD)
Brazil [20]	2007	1433 mother–child pairs	H (mother and children)	Height-to-age, weight-to-age, weight-to-height	–

Turkey [21]	2013.5–2016.12	100 cases, 70 healthy controls	I, M	Neural tube defect	–
Neurodevelopment and behavioral problems					
Taiwan [9]	2004.4–2005.1	266	C	Child Behavior Checklist 1.5/5 (CBCL/1.5 – 5)	+ (negative association)
Faroe Islands [22]	1986–1987	814	C	A panel of neuropsychological tests	+ (negative association)
Ohio, United States [23]	2003.2–2006.1	389	M	NICU Network Neurobehavioral Scale (NNNS)	–
China [24]	2010–2012	410	C, M	Gesell developmental schedules	–
Croatia [25]	2006–2011	198	C	Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III)	+ (negative association)
China [26]	2013–2014	270	H	Bayley Scales of Infant Development, Second Edition (BSID-II)	+ (negative association)
Avon, United Kingdom [27]	1991–1992	2776	M	Strengths and Difficulties Questionnaire	–
Slovenia, Croatia [28]	2006–2011	237 (Slovenia) 124 (Croatia)	C	Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III)	+ (negative association)
Spain [29]	2004–2008	1362	C	McCarthy Scales of Children's Abilities (MSCA)	+
United States [30]	1999–2001	321	C	Cognitive (Peabody Picture Vocabulary Test)	–

(continued)

Table 11.1 (continued)

Location, reference	Year	Sample size	Specimen (I-infant; C-cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (–no or weak association; +association)
Japan [31]	2003–2006	566	C, M, H	Bayley Scales of Infant Development, Second Edition (BSID-II) and Kyoto Scale of Psychological Development (KSPD)	+ (negative association)
Seychelles, Spain, Italy and Greece [32]	2001–2012 (Seychelles) 2003–2009 (Spain) 2006–2011 (Italy and Greece)	1160 (Seychelles) 625 (Spain) 854 (Italy and Greece)	C, H	Bayley Scales of Infant Development (BSID)	+ (CYP 3A gene modification effect)
Korea [33]	2006–2010	458	C, M	Autistic behavior (Social Responsiveness Scale)	+
Korea [34]	2006–2010	251	C	Cognitive development scores	–
Avon, United Kingdom [35]	1991.4–1992.12	2062	H	Intelligence quotient	–
Seychelles [36]	2008–2011	1449	C, M, H	Bayley Scales of Infant and Toddler Development	+ (negative association)
United Kingdom [37]	1991.4–1992.12	1569–2224	M	Scholastic test covering spelling, reading, phoneme understanding, mathematics and science	–
Korea [38]	2006–2010	–	C, M	Bayley Scales of Infant and Toddler Development, Second Edition (BSID-II)	+ (negative association)
Mediterranean (Italy, Slovenia, Croatia, Greece) [39]	2006–2009	1308 mother–child pairs	C, H	Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III)	–

China [40]	2008.9–2009.10	286 mother–infant pairs	C	Neonatal Behavioral Neurological Assessment (NBNA); Bayley Scales of Infants and Toddler Development, Third Edition (BSID-III)	+ (poorer NBNA) − (BSID-III)
Ohio, USA [41]	2003.3–2006.1	389 mother–child pairs	C	Behavioral Assessment system for children (BASC-2) Spence Children's Anxiety Scale (SCAS)	− (BASC-2) + (Anxiety)
Other health outcomes					
United States [42]	–	138	C, maternal toenail	DNA methylation and white blood cell composition of cord blood	+
Hong Kong [43]	2000.7–2001.12	1057	C	Cytokines	+ (interleukin-10)
Faroe Islands [44]	1986–1987, 1997–2000, 2007–2009	2152 mother–child pair	C, M, H	Secondary sex ratio	+ (male ratio)
Seychelles [45]	2001	229	H	Leukocyte telomere length	−
United States [46]	1999–2002	481	C, M	Global DNA methylation and hydroxymethylation	+
Seychelles [47]	2008–2011	1488 mother–child pairs	C, M	Mitochondrial DNA copy number (RmtDNAcn)	+ (maternal blood Hg and higher maternal RmtDNAcn) + (fetal cord blood Hg and lower fetal RmtDNAcn)

11.2 Fetal Growth and Pregnancy Outcome

Birth anthropometry such as weight, length, and head circumference are commonly measured birth outcomes. Most of the studies we reviewed reported adverse anthropometric-related impacts of prenatal environmental mercury exposure [11–15, 18]. Any discrepancies in the findings may have been tied to varying exposure levels or sources. For example, Chinese fish consumers from Yangtze River outlet and Hangzhou Bay Estuary regions reported a negative association, but not other Chinese general population [10, 13]. The modification effect of fish or seafood consumption was also reported by studies in Spain, Korea, and Taiwan [14, 15], while gender differences were reported by [17, 31] as a negative association of THg with birth weight was found only in the male newborns [17]. Two studies had investigated child growth. A study in Chinese fish consumers reported that low-level prenatal Hg exposure could play a role in attenuating infant growth at 12 month old [11]. However, an investigation in Brazil with 1433 mother–infant pairs did not find an influence of maternal fish intake (or its attendant MeHg exposure) on childhood growth until 59 months [20].

Other pregnancy outcomes have not been thoroughly explored. Exposure to Hg during pregnancy was associated with early reductions in biparietal diameter [19]. An association between Hg plasma levels and increased risk for the development of neural tube defects was not observed [21].

11.3 Neurodevelopment and Behavioral Problems

Neurotoxicity remains the major concern of mercury exposure among the developing fetus and children. Nine studies used the Bayley Scales of Infant and Toddler Development (BSID) tool to assess early childhood neurodevelopment. Although 7 out of 9 reported negative associations, the influence of the subscales such as fine motor and cognition was not consistent [17, 25, 26, 28, 29, 31, 32, 36, 38–40, 48]. In the Tohoku Study of Child Development, the boys appeared to be more vulnerable to the exposure than girls [31].

Other studies used different neurodevelopment assessment tools for children from 0 up to 20 years old [22–24, 27, 33–35, 41]. Smaller, but significant, cognitive deficits were observed in association with maternal contaminated seafood in a birth cohort at age 22 years [22]. The impact of prenatal mercury exposure among the same population (cohort study) at different ages was diverse as well. A longitudinal study included 286 mother–child pairs in China revealed only adverse impacts on the Neonatal Behavioral Neurological Assessment, but not on BSID at 18 months of age [40]. The Health Outcomes and Measures of the Environment (HOME) study in Ohio revealed minimal evidence of mercury associated detrimental effects on neurobehavioral outcomes during early infancy and child behavioral from age 2 to

8 years, except for anxiety [23, 41]. The Mothers' and Children's Environmental Health (MOCEH) study in Korea reported adverse effects of prenatal mercury exposure on early neurodevelopment and autistic behaviors in children at 5 years of age, but not cognitive development [33, 34, 38]. Nevertheless, results from the Avon Longitudinal Study of Parents and Children (ALSPAC) did not show adverse effects of maternal total mercury concentrations in blood on child and adolescent behavior nor on child IQ [2, 27, 35]. Interpretation of these studies is difficult, as inorganic mercury only passes the placenta to a very small extent, while methylmercury concentrations are higher in cord blood than in maternal blood.

The beneficial nutrients from fish consumption were proposed by several studies as part of a contribution to the null association between mercury exposure and child neurodevelopment [23, 27, 29, 32, 35, 37]. Llop et al.'s study showed that the relationship between CB-Hg concentrations and child neuropsychological development was influenced by maternal nutritional factors, such as fish consumption and the polyunsaturated fatty acid (PUFA) status [29]. Meanwhile, the Eastern Mediterranean study also reported inconsistent results on the subscales of child neurodevelopment [39]. Yet, interpretation with caution is needed due to different mercury form measured. Another factor proposed to affect individual variability to detrimental mercury action are genetic polymorphisms. 247 SNPs were studied in sub-sample of ALSPAC. Although the neuropsychological adverse impact of low-level MeHg exposure was not obvious, MeHg interaction was detected for rs662 (Paraoxonase 1), rs1042838 (Progesterone Receptor), rs3811647 (Transferrin), and rs2049046 (Brain-Derived Neurotrophic Factor) [49]. The modification effect of Apolipoprotein-E (APOE), Paraoxonase 1 gene (PON1), cytochrome p450 3A (CYP3A) families was reported [9, 28, 30]. In addition, maternal polymorphisms in glutathione-related genes are found to be associated with maternal mercury concentrations and early child neurodevelopment in a population with a fish-rich diet [36, 48].

11.4 Other Health Effects

There were not many studies investigating other health effects of prenatal mercury exposure.

Sex ratios related to prenatal MeHg exposure had been investigated by a study combining three Faroese birth cohorts and revealed a doubling in mercury concentrations was associated with increased odds by 9% (95% CI 2–17%) of giving birth to a boy [44]. Cytokine profiles had been measured to explore mercury immunotoxicity among 1057 children aged between 6 and 9 years. Childhood, but not fetal mercury exposure, was negatively associated with childhood IL-10 levels [43].

Recent advances in the fields of genomics and bioinformatics have implied the possibility of epigenetic modification by environmental toxicants. Epigenetics

explores heritable changes in gene expression but not involved in changes in DNA sequence, and DNA methylation is one of the epigenetic mechanism. Studies by Cardenas et al. [42, 46] had revealed different DNA methylation patterns in cord blood of infants exposed to prenatal mercury, in which the methylation changes persisted in early but not middle childhood blood [42, 46]. A survey from the Seychelles Child Development Study had measured leukocyte telomere length (LTL), aging related biomarker, and did not find evidence of MeHg involvement [45]. Another investigation in Seychelles island found small association between maternal blood and cord blood mercury and relative mitochondrial DNA (mtDNA) copy number changes [47]. mtDNA copy number is related to mitochondrial function for providing energy to cells including nervous system. There is not yet a convincing relationship between the aforementioned biomarkers, MeHg exposure, and adverse health impact. Nevertheless, this new field could have potential to elucidate the individual variability and pathogenesis of mercury toxicity in the future.

11.5 Lead

Exposure to lead was first documented in the 1970s. Lead, a neurotoxin could accumulate in soft tissues and bones causing neurological disorders related to decreased attention and other cognitive functions and behavioral problems. By the mid-1980s, lead use in the environment had declined. Environmental laws had been established to regulate the use of lead in gasoline, paints, solders, and water systems. Yet, lead exposure still remains and attracts public concern.

Since the 1970s, the USA has made progress in lowering children's blood lead levels. Reduction in lead exposure ranks as one of the ten greatest public health achievements in recent history [50]. Blood lead levels have declined by more than 90 percent since the mid-1970s. The four goals of the Lead Action Plan were announced on March 2019 to reduce exposure to lead and improve children's health strategically and collaboratively. In the prenatal period, the heavy metal level would affect fetal development. The children who were exposed to low doses of lead are particularly with neurotoxicity. Even if the blood lead levels are lower than the public health threshold of 100 μg/L, the neurodevelopment in children can still be affected [51]. The Centers for Disease Control and Prevention (CDC) in the USA has set the reference concentration of lead in blood at 50 g/L for children. There is no known threshold for Pb and even low-level exposure has been shown to affect children's health. The best way to reduce blood lead levels is to address all sources of Pb exposure.

Lead is a neurotoxin that affects the central nervous system (CNS); specifically, lead affects cognitive functions, hearing, and posture of children. Lead can accumulate in body tissues, especially bones [52]. In utero is particularly high risk to lead exposure. Maternal B-Pb can readily cross the placenta and enter fetal blood and affect the health status [53]. The summary of Pb birth cohort studies is shown in Table 11.2 and details are described as follows.

Table 11.2 Association between lead (Pb) and children's health outcomes based on cohort study

Location, reference	Year	Sample size	Specimen (I-infant; C-cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (–no or weak association; +association)
Fetal growth and pregnancy outcome					
USA [54]	1999–2002	2,128	Maternal blood	Birth outcomes	+
Japan [55]	2010–2012	602	Maternal blood	Birth weight	+
Saudi Arabia [56]	2012–2014	120	Maternal blood	Preeclampsia	+
Korea [57]	2006–2010	1751	Children's blood	K-WPPSI (Korean version of the Wechsler Preschool and Primary Scale of Intelligence)	+
UK [58]	1991–1992	4285	Maternal blood	Birth outcome	+
UK [59]	1991–1992	14,541	Maternal blood	Birth outcomes	+
UK [59]	1991–1992	4190	Maternal blood	Birth outcome Pregnancy outcomes Questionnaires	+
Mexico [60]	2007–2011	944	Maternal blood	Gestational age birth weight	+
Mexico [61]	2007–2011	1054	Maternal blood	Children's growth parameters	+
USA [62]	2011–2015	156	Maternal blood	Teeth	+
China [63]	2009	3125	Maternal blood	Birth outcome	+
China [64]	2009	3125	Maternal blood	Birth outcome	+
China [65]	2012–2014	11,311	Maternal blood and urine	Gestational age	+
China [66]	2012–2014	7290	Maternal urinary	Premature rupture of membranes, PROM	+
Mexico [67]	2007–2011	565	Maternal blood	Children's resting blood pressure	+

(continued)

Table 11.2 (continued)

Location, reference	Year	Sample size	Specimen (I-infant; C-cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (–no or weak association; +association)
Neurodevelopment and behavioral problems					
Nepal [68]	2008	119	Cord blood	Brazelton Neonatal Behavioral Assessment Scale, Third Edition (NBAS III), the Bayley Scales of Infant Development, Second Edition, (BSID II)	+
China [69]	2009–2010	362	Cord blood Infants blood	Bayley Scales of Infant Development (BSID-II)	+
China [69]	2009–2010	415	Maternal blood	Neonatal behavioral neurological assessments (NBNA)	+
Sweden [70]	1978–2007	3176	Children's blood	School performance	+
USA [71]	1994–1998	58,650	Children's blood	3rd grade ISAT scores, a measure of individual student achievement relative to the Illinois Learning Standard	+
Mexico [72]	1994–2011	192	Maternal bone Children's blood	The Coopersmith Self-esteem Inventory (Spanish version, adult short form) Childhood ADHD symptoms Spanish versions of Conners' Parental Rating Scale-Revised (CPRS-R) Behavior Rating Inventory of Executive Function-Parental (BRIEF-P) Form	+

Korea [73]	2006–2010	965	Cord blood Maternal blood	Korean version of Bayley Scales Infant Development II (K-BSID-II)	+
Korea [74]	2006–2009	Among 1286	Maternal blood	Psychosocial Well-Being Index Short Form (PWI-SF) Center for Epidemiological Studies Depression Scale (CES-D) The Korean Bayley Scales of Infant Development-II assessment (BSID-II) the standardized mental development index (MDI) psychomotor developmental index (PDI) Korean Ages and Stages Questionnaires (K-ASQ)	+
China [75]	2010–2012	225	Maternal blood	Symptom Checklist-90-Revised (SCL-90-R) Gesell Development Scale	+
USA [76]	2017	4484	Maternal blood Prenatal blood	Wechsler Preschool and Primary Scale of Intelligence—Revised UK edition (WPPSI), Wechsler Intelligence Scale for Children WISC-III UK	+
USA [77]	2012–2013	208	Children's blood	Body mass index the impulsivity scale of the Child Behavior Checklist Anxiety or depression Child Behavior Checklist scale	+

(continued)

Table 11.2 (continued)

Location, reference	Year	Sample size	Specimen (I-infant; C-cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (–no or weak association; +association)
New Zealand [78]	1972–1973	1037	Children's blood	Wechsler Adult Intelligence Scale –IV (WAIS-IV)	+
USA [79]	2016	1479	Maternal blood Children's blood	ADHD diagnosis	+
Other health outcomes					
USA [80]	2009–2011	321	Maternal blood Cord blood	DNA methylation	+
Russia [81]	2003–2005	516	Children's blood	Growth, pubertal onset and sexual maturity	+
Kosovo [82]	1984–1985	101	Maternal blood	sICAM-1 and sVCAM-1	+
USA [83]	2009	323	Maternal blood	Child's height and weight blood pressure	+

11.6 Fetal Growth and Pregnancy Outcomes

As well-recognized, lead is a toxic heavy metal widely present in the environment. Although, due to public concern, lead exposure has declined in recent decades, toxic effects still have health impacts especially among sensitive populations, such as the developing fetus and children. Many of the studies revised investigated higher prenatal and early postnatal lead exposure and its contribution to a higher risk of having a small-for-gestational age (SGA) infant and lower birth weight even during the first trimester. Blood lead levels (B-Pb), once believed to be safe under 5 μg/L, were found, in some studies, at an increase of 1 μg/L to be associated with a birth weight of −9.93 (95% CI 20.27, 0.41) g, head circumference −0.03 (95% CI 0.06, 0.00) cm, and crown–heel length −0.05 (95% CI 0.10, 0.00) cm [60]. The prenatal lead exposure reduced birth weight and other birth outcomes especially in male offspring.

Preeclampsia is the greatest threat to maternal morbidity and mortality. The increasing levels of serum lead was observed to pose a significant risk in pregnant women to preeclampsia was observed [56]. Premature rupture of membranes (PROM) is the spontaneous rupture of amniotic membranes before the onset of labor which are related to maternal fetal and neonatal risks. Higher levels of maternal Pb exposure increased the risk of PROM, particularly preterm birth among boys [66].

11.7 Neurodevelopment and Behavioral Problems

Early childhood Pb exposure is associated with poorer achievement. For prenatal exposure to lead, the higher exposure resulted in poorer achievement on standardized reading and math tests and higher incidence of the attention-deficit hyperactivity disorder (ADHD). Boys were found more vulnerable to higher exposures than girls [79]. In Korea, researchers found that Pb levels during late pregnancy were significantly associated with increased risk of behavioral problems in boys [84]. But, reduced prenatal lead exposure with adequate iron intake showed better neurodevelopment outcomes. Another finding showed that prenatal maternal stress and depressive symptoms, and mental development index (MDI) scores in 6-month-old infants after adjustment for prenatal lead exposure affected cognitive function negatively [74]. In a China study, prenatal and postnatal lead exposures as low as 5 mg/dl were found to have an adverse effect on neurodevelopment [69]. However, lead causes neurotoxic effects at very low exposures (B-Pb < 50 mg/L) in childhood and it was also reported that these effects remain for many years [70]. Results from our review of Pb's impact on fetal and early childhood neurodevelopment consistently showed that Pb has the potential to cause enduring, neurotoxic effects at very low exposures (B-Pb < 50 mg/L).

11.8 Other Health Effects

Evidence shows that environmental lead exposures can have negative influences over the course of a lifetime. Some metal exposures may be associated with early life increases in blood pressure in children with the capability to lead to long-term health effect. Pb also has been shown to interfere with many aspects of hormonal action and may delay the timing of male puberty [81].

The soluble vascular adhesion molecule (sVCAM-1) and soluble intercellular cell adhesion molecule (sICAM-1) are the biomarker of systemic inflammation and endothelial dysfunction. One study observed that the blood lead concentration is associated with the blood pressure and also is associated with the serum levels of markers of sVCAM-1 and sICAM-1 [82].

As known, in vitro and in vivo exposure to toxic could alter DNA methylation. The animal study found the Pb exposure alters DNA methylation in human [85]. One study also found that prenatal Pb exposure alters DNA methylation of imprinted genes resulting in lower birth weight and rapid growth. Imprinting is established early and maintained via DNA methylation that prenatal Pb levels in the upper tertile had higher methylation of the regulatory region of the MEG3 DMR imprinted domain and associated with lower birth weight and rapid gains in adiposity by age 2–3 years [80].

11.9 Manganese (Mn)

For humans, manganese (Mn) is considered one of the naturally essential elements and comprises about 0.1% of the earth's crust; however, it becomes toxic in excess. As an essential element, Mn plays a pivotal role in maintaining normal function such as formation of cartilage, wound-healing, and urea cycle. Inorganic and organic forms of Mn exist in the environment, inorganic Mn is an ingredient in wide range of industrial processes and commercial products such as steel and aluminum alloy formation and production; the organic is used as fungicide, additives, and medical use.

The main sources of Mn are derived from the environment and food supply. Air, soil, and water are the pathways from the environment, and rice, seafood, fruits, and vegetables are the daily intake. The Food and Nutrition Board (FNB) of the Institute of Medicine (IOM) has set Mn adequate intake (AI) levels for human, such as the AI for pregnant women is 2.0 mg/day; for infants between 0 and 6 months is 0.003 mg/day. The European Food Safety Authority (EFSA) set a daily dose of 3.0 mg/day for adults including pregnant women. Mn has a longer half-life in women than in men [86]. Therefore, establishing a Mn requirement on human should differentiate by gender, but individual variation also may exist.

Excessive or inadequate intake of Mn impacts human health. Manganese deficiency may interfere with body function; however, this is not clearly understood.

Adverse health effects of high level Mn exposures were first noted associated with inhalation in occupational settings, and the effect of Mn exposure by inhalation was observed due to direct transport to brain without metabolization. Oral exposure from Mn contaminated drinking water also showed similar symptoms such as tremors, difficulty walking, and facial muscle spasms which may appear slowly later in life. To date, permanent neurological disorder has been recognized as an adverse health effect of excessive Mn exposure [86].

Mn is mainly excreted in the bile, and much less via urine. Although gender differences exist, normal range of Mn elimination in blood is 4–15 μg/L, in urine is1–8 μg/L, and in serum is 0.4–0.85 μg/L. In addition to the neurological effects, developmental, respiratory, and reproductive effects also have been reported in relation to Mn exposure. Excessive exposure to Mn effects neuro or behavior functions in children and adults, especially in children during critical developmental periods. High level of Mn in drinking water in Quebec reported that hair Mn in school-age students was associated with behavioral problems [87]. The other study in Italy for high level of Mn exposure reported that olfactory and motor function changes in adolescents were associated with blood and hair Mn [88]. Excessive or insufficient Mn exposure is a public health concern for childhood development. For example, Mn exposure has been found to be associated with anxiety and motor impairment [89]. Also, in infants and children, Mn exposure can be particularly detrimental as their organ systems are immature and are, thus, easy targets for Mn exposure. Mn also accumulates greatly in infants and children through maternal exposure during pregnancy [90]. Although many epidemiologic and animal data have reported the adverse effects of high Mn exposure, the cohort studies of Mn exposure effects are few. Therefore, Table 11.3 presents the recent years evidences of birth cohort studies and includes the health effects regarding fetal growth and pregnancy outcome, and neurodevelopment and behavioral problems, and the detail described as follows.

11.10 Fetal Growth and Pregnancy Outcome

Literature on the relationship between Mn and pregnancy outcomes in humans is very sparse, but pregnant women exposed to Mn may have toxic effects on the developing fetus. Gestational age is a non-invasive measurement for evaluation of most effects, but only two studies considered to explore the association between Mn level and pregnancy outcome. Birth weight, on the other hand, is one of the key indicators to evaluate the fetal and neonatal health condition. An important cause of perinatal morbidity and mortality is fetal growth restriction including environmental exposure, low pregnant weight, poor maternal nutrition, etc. Most studies have found an association between birth weight and Mn exposure by different biomarkers, and some studies also have considered birth length, head circumference, and chest circumference for exploration. However, some of these cohort studies examined populations with likely high Mn exposures, for example, due to living near industry, so the Mn levels were higher than the general population. Mora et al. [114]

Table 11.3 Association between manganese (Mn) and children's health outcomes based on cohort study

Location, reference	Year	Sample size	Specimen (I-infant; C-Cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (−no or weak association; +association)
Fetal growth and pregnancy outcome					
Iran [91]	2006.10–2009.2	364	M	Gestational hypertension	+
China [92]	2008–2009	1377	M and C	Ponderal index	+
Korea [93]	2007.7–2009.12	331	M	Birth weight	+
China [94]	2006.9–2007.4	172	M and C	Birth weight	+
China [95]	2006.10–2007.3	125	M and C	Birth weight, birth height, head circumference, chest circumference	+
USA [96]	2010.3–2011.6	449	M and W and H	Gestational age	+
Taiwan [97]	2010.3–12	168	M	Birth weight, length, head circumference, and chest circumference	+
Bangladesh [98]	2001.11–2003.10	758	W and M	Birth weight, birth length	+
USA [99]	2010.3–2011.6	331	M and H	Birth weight, body length, head circumference, chest circumference, length of gestation	+
Iran [100]	2012.12–2013.6	1033	Food frequency questionnaire	Preterm delivery	+
China [101]	2012.11–2014.4	204 LBW cases and 612 control	U	Birth weight	+
Canada [102]	2008–2011	1938	M and C	Food frequency questionnaire	+
Canada [103]	2008–2011	1983	M and C	Birth weight	+

Bangladesh [104]	2008–2011	764	C	Birth weight, length, head circumference, and cognitive development	+
Japan [105]	2011.2–2014.03	14847	M	Preterm birth	–
Mexico [106]	1997–2004	250	U	BMI z-score	+
Neurodevelopment and behavioral problems					
Mexico [107]	1997–2000	448	I	Bayley-II	+
Taiwan [108]	2004.4–2005.1	230	C	Comprehensive developmental inventory for infants and toddlers (CDIIT)	+
China [109]	2008–2009	933	C	Neonatal Behavioral Neurological Assessments (NBNA)	+
USA [110]	2008–2011	17000	W	Delayed milestones, speech/language disorder, and hearing loss	+
Sweden [111]	1978–2000	419	C	Attention deficit hyperactivity disorder (ADHD)	–
USA [112]	1999—2000	601	M and teeth	Bayley-II	+
Korea [113]	2007–2011	232	M	Scales of Infant Development-II (BSID-II)	+
USA [114]	2009.9–2011.8	248	Teeth	ADHD, DSM-IV, CPT-II, Behavior Assessment System for Children, Second edition (BASC-2)	+
China [115]	2010.9–2013.12	774	C	Neurodevelopment quotient	+
Bangladesh [116]	2008–2011	524	W	Bayley Scales of Infant Development-III (BSID-III)	+

(continued)

Table 11.3 (continued)

Location, reference	Year	Sample size	Specimen (I-infant; C-Cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (−no or weak association; +association)
Bangladesh [117]	2002.10–2003.12	1265	W	Wechsler Intelligence Scale for Children (WISC-IV), Strengths and Difficulties Questionnaire (SDQ)	+
USA [118]	2002–2011	224	M and C	Bayley-II	+
Bangladesh [119]	2010–2013	825	C	Bayley-III	+
USA [120]	2013.3–2014.6	106	H	Full Scale IQ	+
Italy [48]	2010–2014	195	Teeth	SNPs associated with development	+
Spain [121]	2000–2008	302	Placenta	McCarthy Scales of Children's Abilities (MSCA)	+
Mexico [122]	2007–2011	473	M and C	Bayley-III	+
Costa Rica [123]	2010.3–2011.6	355	H and M	Bayley Scales of Infant Development-III (BSID-III)	+

reported the mean concentration of Mn in blood during pregnancy was 24.5 μg/L and found no association, while a positive association was found between maternal hair Mn level within 3.5 μg/g [114]. Combined indicators also were widely used in exploration of exposure assessment such as intrauterine growth retardation (IUGR), Ponderal index, or body mass index (BMI). These recent studies indicated a higher or lower Mn level was associated with fetal growth and pregnant outcomes; however, further analysis is needed to clarify these associations.

11.11 Neurodevelopment and Behavioral Problems

Clear evidence indicates that human exposure to Mn is associated with neurological effects. Mn easily crosses the blood–brain barrier in the developing fetus and neonate. Neurological effects can be generally classified into three categories: mental, behavioral, and motor function effects. IQ was evaluated to identify as children's mental effects. Haynes et al. recruited children 7–9 years of age, living near a hazardous waste incinerator and Mn processor in East Liverpool, Ohio, and collected their blood and hair. The researchers found that these children poorly performed on Full Scale IQ [36, 48]. It is possible that airborne Mn concentrations have exceeded US EPA reference levels for decades. Poor behavioral problems have been reported by measured Mn levels in prenatal and early postnatal dentine of shed teeth in 248 school-age children revealing the long-term effects of Mn [99]. On the other hand, a study examining 232 pairs of pregnant women and their infants at 6 months of age found maternal blood manganese and psychomotor development indexes for children had an inverted U-shape dose–response curve [113].

Based on available evidence, higher levels of Mn are associated with developmental and neurological effects [124]. The varied tools for neurological evaluation have been developed for different uses but all for children's health. New biomarkers such as teeth also have shown to be a new indicator for internal dose evaluation for Mn levels in body. On the other hand, interaction between other metals and Mn also is a noteworthy assessment of the health hazards of Mn exposure. Therefore, Mn as one of the world's most abundant metals is a worldwide problem, especially in highly polluted areas. It is important to prevent pregnant women and children from higher level Mn exposure.

11.12 Other Health Effects

Although most current evidence demonstrates neurological and developmental effects of Mn exposure, some studies have found an association with respiratory or reproductive effects. Inhalation exposure to Mn has been shown to arouse an inflammatory response in the lung; however, this observation was reported in the workplace rather than in the general environment. Even so, air contaminated with Mn

from factory or occupational settings or other sources may be of concern. In addition, male workers who have been exposed to Mn for 1–19 years had been reported to have impaired fertility [125], but not for female workers. Some evidence has shown an association of Mn with respiratory and reproductive effects in rodents; however, further evidence from epidemiological studies are needed to better delineate the impact of Mn on the general, as well as vulnerable, populations.

11.13 Vanadium (V) and Thallium (Tl)

Vanadium (V) and thallium (Tl) have aroused public attention recently. Vanadium (V) is one of the abundant transition metals and has been widely used in modern industry. The burning of crude oil, coal, and combustion of petroleum fuels will release it into the environment air, soil, and water and distributed into the human body by attaching the particles matter [126]. V can cross the placental barrier, exposing the developing fetus and accumulating in the fetal skeleton [127]. V has been classified as a possible carcinogen for humans by International Agency for Research on Cancer (IARC). Deficiencies in V result in reduced growth in rats. Animal studies have shown that exposure to V during pregnancy induces reproductive toxicity and affects the development and behavior of offspring. However, human studies only showed little evidence on adverse birth outcomes (Table 11.4).

Tl is widely used in electronic equipment, semiconductor, optical lenses, semiconductors, scintillation counters, chemical catalysts, crystals, and many manufacturing industries and known as one of the hazardous toxic heavy metals [134]. Mining activities, coal and oil combustion, cement plants, and refining processes release Tl. The Tl distributed ways were similar to the other heavy metal, and human's nervous system, lung, heart, liver, and kidney can be affected by exposure to air, water, and food with large amounts [135]. Industrial workers and general population exposure to high level of Tl has affected human health [136]. Tl can also cross the placental barrier, potentially eliciting embryonic toxicity or genotoxicity in animal studies. High level Tl exposure from industrial pollution during pregnancy was associated with fetal death, prematurity, or decreased birth weight [137]. Epidemiology studies have indicated that exposure of this specific heavy metal may result in adverse birth outcomes, but the exposure of this rare metal needs to be better studied.

11.14 Fetal Growth and Pregnancy Outcome

Table 11.4 describes more details of V and Tl on fetal growth and pregnancy outcomes. One study indicated that pregnant women exposed to higher levels of V might lead to an increased risk of PROM [79]. Adjusted OR of 1.57 for PROM was observed with one unit increase in natural logarithmically transformed urinary V

Table 11.4 Association between vanadium (V) and thallium (Tl) and children's health outcomes based on cohort study

Location, reference	Year	Sample size	Specimen (I-infant; C-Cord blood; M-maternal; U-urine; H-hair; W-water)	Main outcome	Association (−no or weak association; +association)
Fetal growth and pregnancy outcome					
China [128]	2012–2014	816	(V) Maternal urine	Birth weight	+
China [63]	2012–2014	7297	(V) Maternal urine	Birth weight Gestational age	+
China [129]	2012–2014	11,311	(V) Maternal urine	PROM is defined as spontaneous membrane rupture before the onset	+
China [130]	2013–2016	3075	(V) Maternal urine	fetal growth and birth size	+
China [101]	2012–2014	816	(Tl) Maternal urine	Birth weight Gestational age	+
China [131]	2012–2014	7173	(Tl) Maternal urine	Gestational age	+
China [132]	2013–2014	3236	(Tl) Maternal blood Umbilical cord blood	Birth outcomes	+
China [133]	2013–2014	3080	(Tl) Maternal blood Cord blood	Z-scores for weight-for-age (WAZ), height-for-age (HAZ), weight-for-height (WHZ)	+

concentration. Another Chinese Cohort study found newborns' birth size was restricted and associated with higher V exposure in the first, early second, and late third trimesters [130], suggesting that critical windows of fetal growth, during three trimesters, are more vulnerable to V. For a case–control study, the low birth weight mothers observed had significantly higher urinary V levels compared to the control mothers [median: 1.27 mg/l (3.04 mg/g creatinine) versus 1.22 mg/l (1.93 mg/g creatinine)] [128]. Even when stratified by maternal age and infant gender, a similar trend remained. On the other hand, urinary Ln-V concentrations showed nonlinear dose–response relationships with risk of preterm delivery and low birth weight [63].

Higher maternal Tl concentration was significantly associated with increased risk of low birth weight for OR 1.90 and 3.58 for the highest versus lowest tertile [101]. Prenatal Tl exposures may have a sex specific effect on child anthropometric measurements in the first 2 years of life [133]. The cord blood Tl levels showed tended to be associated with reduced child's stature and weight in young girls.

11.15 Summary

Metals are part of the natural environment. The balance of contribution to modern life convenience and toxic threats to pregnant women and children is crucial. Neurotoxicity is still the major concern of MeHg and Pb exposure even at environmentally exposed low levels. Exposure should be minimized especially for susceptible population. However, the safety margin for Mn intake should be estimated for the nonlinear dose–response curve between Mn levels and neurobehavioral problems. As new emerging hazards, only limited studies support the adverse impact of V and Tl on fetal growth and pregnant outcomes.

In order to further promote child health, more researchers, as well as state-of-the-art technology to investigate individual vulnerability and pathogenesis of toxicity, are warranted. Risk communication also is vital to ensure the importance of adequate nutrition intake while avoiding environmental pollutants.

References

1. National Academy of Sciences. Methylmercury, toxicological effects of methylmercury. Washington: National Academies Press; 2000.
2. Groth E 3rd. Ranking the contributions of commercial fish and shellfish varieties to mercury exposure in the United States: implications for risk communication. Environ Res. 2010;110(3):226–36.
3. Sharma BM, Sáňka O, Kalina J, Scheringer M. An overview of worldwide and regional time trends in total mercury levels in human blood and breast milk from 1966 to 2015 and their associations with health effects. Environ Int. 2019;125:300–19.
4. Nakayama SF, Iwai-Shimada M, Oguri T, Isobe T, Takeuchi A, Kobayashi Y, et al. Blood mercury, lead, cadmium, manganese and selenium levels in pregnant women and their deter-

minants: the Japan Environment and Children's Study (JECS). J Expo Sci Environ Epidemiol. 2019;29:633–47. https://doi.org/10.1038/s41370-019-0139-0.
5. Ursinyova M, Masanova V, Uhnakova I, Murinova LP, Patayova H, et al. Prenatal and early postnatal exposure to total mercury and methylmercury from low maternal fish consumption. Biol Trace Elem Res. 2018;191:16–26. https://doi.org/10.1007/s12011-018-1585-6.
6. Axelrad DA, Bellinger DC, Ryan LM, Woodruff TJ. Dose-response relationship of prenatal mercury exposure and IQ: an integrative analysis of epidemiologic data. Environ Health Perspect. 2007;115(4):609–15.
7. World Health Organization. International Programme on Chemical Safety|Mercury. https://www.who.int/ipcs/assessment/public_health/mercury/en/. Accessed 29 April 2019.
8. Andreoli V, Sprovieri F. Genetic aspects of susceptibility to mercury toxicity: an overview. Int J Environ Res Public Health. 2017;14(1):93.
9. Ng S, Lin CC, Jeng SF, Hwang YH, Hsieh WS, Chen PC. Mercury, APOE, and child behavior. Chemosphere. 2015;120:123–30.
10. Hu X, Zheng T, Cheng Y, Holford T, Lin S, Leaderer B, et al. Distributions of heavy metals in maternal and cord blood and the association with infant birth weight in China. J Reprod Med. 2015;60(1–2):21–9.
11. Ou L, Chen C, Chen L, Wang H, Yang T, Xie H, et al. Low-level prenatal mercury exposure in North China: an exploratory study of anthropometric effects. Environ Sci Technol. 2015;49(11):6899–908.
12. Thomas S, Arbuckle TE, Fisher M, Fraser WD, Ettinger A, et al. Metals exposure and risk of small-for-gestational age birth in a Canadian birth cohort: the MIREC study. Environ Res. 2015;140:430–9.
13. Tang M, Xu C, Lin N, Liu K, Zhang Y, Yu X, et al. Lead, mercury, and cadmium in umbilical cord serum and birth outcomes in Chinese fish consumers. Chemosphere. 2016;148:270–5.
14. Murcia M, Ballester F, Enning AM, Iñiguez C, Valvi D, Basterrechea M, et al. Prenatal mercury exposure and birth outcomes. Environ Res. 2016;151:11–20.
15. Kim BM, Chen MH, Chen PC, Park H, Ha M, Kim Y, et al. Path analysis of prenatal mercury levels and birth weights in Korean and Taiwanese birth cohorts. Sci Total Environ. 2017;605–606:1003–10.
16. Valvi D, Oulhote Y, Weihe P, Dalgård C, Bjerve KS, et al. Gestational diabetes and offspring birth size at elevated environmental pollutant exposures. Environ Int. 2017;107:205–15.
17. Tatsuta N, Kurokawa N, Nakai K, Suzuki K, Iwai-Shimada M, Murata K, et al. Effects of intrauterine exposures to polychlorinated biphenyls, methylmercury, and lead on birth weight in Japanese male and female newborns. Environ Health Prev Med. 2017;22(1):39.
18. Vigeh M, Nishioka E, Ohtani K, Omori Y, Matsukawa T, Koda S, et al. Prenatal mercury exposure and birth weight. Reprod Toxicol. 2018;76:78–83.
19. Ballester F, Iñiguez C, Murcia M, Guxens M, Basterretxea M, Rebagliato M. Prenatal exposure to mercury and longitudinally assessed fetal growth: relation and effect modifiers. Environ Res. 2018;160:97–106.
20. Cunha MPL, Marques RC, Dórea JG. Influence of maternal fish intake on the anthropometric indices of children in the Western Amazon. Nutrients. 2018;10(9):e1146.
21. Demir N, Başaranoğlu M, Huyut Z, Değer İ, Karaman K, Şekeroğlu MR. The relationship between mother and infant plasma trace element and heavy metal levels and the risk of neural tube defect in infants. J Matern Fetal Neonatal Med. 2019;32(9):1433–40.
22. Debes F, Weihe P, Grandjean P. Cognitive deficits at age 22 years associated with prenatal exposure to methylmercury. Cortex. 2016;74:358–69.
23. Xu Y, Khoury JC, Sucharew H, Dietrich K, Yolton K. Low-level gestational exposure to mercury and maternal fish consumption: associations with neurobehavior in early infancy. Neurotoxicol Teratol. 2016;54:61–7.
24. Hu Y, Chen L, Wang C, Zhou Y, Zhang Y, Wang Y, et al. Prenatal low-level mercury exposure and infant neurodevelopment at 12 months in rural northern China. Environ Sci Pollut Res Int. 2016;23(12):12050–9.

25. Prpić I, Milardović A, Vlašić-Cicvarić I, Špiric Z, Radić Nišević J, et al. Prenatal exposure to low-level methylmercury alters the child's fine motor skills at the age of 18 months. Environ Res. 2017;152:369–74.
26. Rothenberg SE, Yu X, Liu J, Biasini FJ, Hong C, Jiang X, et al. Maternal methylmercury exposure through rice ingestion and offspring neurodevelopment: a prospective cohort study. Int J Hyg Environ Health. 2016;219(8):832–42.
27. Golding J, Gregory S, Emond A, Iles-Caven Y, Hibbeln J, Taylor CM. Prenatal mercury exposure and offspring behaviour in childhood and adolescence. Neurotoxicology. 2016;57:87–94.
28. Tratnik JS, Falnoga I, Trdin A, Mazej D, Fajon V, et al. Prenatal mercury exposure, neurodevelopment and apolipoprotein E genetic polymorphism. Environ Res. 2017;152:375–85.
29. Llop S, Ballester F, Murcia M, Forns J, Tardon A, et al. Prenatal exposure to mercury and neuropsychological development in young children: the role of fish consumption. Int J Epidemiol. 2017;46(3):827–38.
30. Cardenas A, Rifas-Shiman SL, Agha G, Hivert MF, Litonjua AA, et al. Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. Sci Rep. 2017;7(1):288.
31. Tatsuta N, Murata K, Iwai-Shimada M, Yaginuma-Sakurai K, Satoh H, Nakai K. Psychomotor ability in children prenatally exposed to methylmercury: the 18-month follow-up of Tohoku study of child development. Tohoku J Exp Med. 2017;242(1):1–8.
32. Llop S, Tran V, Ballester F, Barbone F, Sofianou-Katsoulis A, Sunyer J, et al. CYP3A genes and the association between prenatal methylmercury exposure and neurodevelopment. Environ Int. 2017;105:34–42.
33. Ryu J, Ha EH, Kim BN, Ha M, Kim Y, Park H, et al. Associations of prenatal and early childhood mercury exposure with autistic behaviors at 5years of age: the mothers and Children's environmental health (MOCEH) study. Sci Total Environ. 2017;605–606:251–7.
34. Lee H, Park H, Ha E, Hong YC, Ha M, Park H, et al. Stability of cognitive development during the first five years of life in relation to heavy metal concentrations in umbilical cord blood: Mothers' and Children's environmental health (MOCEH) birth cohort study. Sci Total Environ. 2017;609:153–9.
35. Golding J, Hibbeln JR, Gregory SM, Iles-Caven Y, Emond A, Taylor CM. Maternal prenatal blood mercury is not adversely associated with offspring IQ at 8 years provided the mother eats fish: a British prebirth cohort study. Int J Hyg Environ Health. 2017;220(7):1161–7.
36. Wahlberg K, Love TM, Pineda D, Engström K, Watson GE, Thurston SW, et al. Maternal polymorphisms in glutathione-related genes are associated with maternal mercury concentrations and early child neurodevelopment in a population with a fish-rich diet. Environ Int. 2018;115:142–9.
37. Hibbeln J, Gregory S, Iles-Caven Y, Taylor CM, Emond A, Golding J. Total mercury exposure in early pregnancy has no adverse association with scholastic ability of the offspring particularly if the mother eats fish. Environ Int. 2018;116:108–15.
38. Kim Y, Ha EH, Park H, Ha M, Kim Y, Hong YC, et al. Prenatal mercury exposure, fish intake and neurocognitive development during first three years of life: prospective cohort mothers and Children's environmental health (MOCEH) study. Sci Total Environ. 2018;615:1192–8.
39. Barbone F, Rosolen V, Mariuz M, Parpinel M, Casetta A, Sammartano F, et al. Prenatal mercury exposure and child neurodevelopment outcomes at 18 months: results from the Mediterranean PHIME cohort. Int J Hyg Environ Health. 2019;222(1):9–21.
40. Wang J, Wu W, Li H, Cao L, Wu M, Liu J, et al. Relation of prenatal low-level mercury exposure with early child neurobehavioral development and exploration of the effects of sex and DHA on it. Environ Int. 2019;126:14–23.
41. Patel NB, Xu Y, McCandless LC, Chen A, Yolton K, Braun J, et al. Very low-level prenatal mercury exposure and behaviors in children: the HOME study. Environ Health. 2019; 18(1):4.
42. Cardenas A, Koestler DC, Houseman EA, Jackson BP, Kile ML, et al. Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. Epigenetics. 2015;10(6):508–15.

43. Hui LL, Chan MHM, Lam HS, Chan PHY, Kwok KM, Chan HIS, et al. Impact of fetal and childhood mercury exposure on immune status in children. Environ Res. 2016;144(Pt A):66–72.
44. Timmermann CAG, Choi AL, Petersen MS, Nielsen F, Budtz-Jørgensen E, Weihe P, et al. Secondary sex ratio in relation to exposures to polychlorinated biphenyls dichlorodiphenyl dichloroethylene and methylmercury. Int J Circumpolar Health. 2017;76(1):1406234.
45. Yeates AJ, Thurston SW, Li H, Mulhern MS, McSorley EM, Watson GE, et al. PUFA status and methylmercury exposure are not associated with leukocyte telomere length in mothers or their children in the Seychelles child development study. J Nutr. 2017;147(11):2018–24.
46. Cardenas A, Rifas-Shiman SL, Godderis L, Duca RC, Navas-Acien A. Prenatal exposure to mercury: associations with global DNA methylation and Hydroxymethylation in cord blood and in childhood. Environ Health Perspect. 2017;125(8):087022.
47. Xu Y, Wahlberg K, Love TM, Watson GE, Yeates AJ, Mulhern MS, et al. Associations of blood mercury and fatty acid concentrations with blood mitochondrial DNA copy number in the Seychelles child development nutrition study. Environ Int. 2019;124:278–83.
48. Wahlberg K, Arora M, Curtin A, Curtin P, Wright RO, Smith DR, et al. Polymorphisms in manganese transporters show developmental stage and sex specific associations with manganese concentrations in primary teeth. Neurotoxicology. 2018;64:103–9.
49. Julvez J, Smith GD, Golding J, Ring S, Pourcain BS. Gonzalez JR, et al. prenatal methylmercury exposure and genetic predisposition to cognitive deficit at age 8 years. Epidemiology. 2013;24(5):643–50.
50. U.S. Centers for Disease Control and Prevention (CDC). 2011. https://www.cdc.gov/mmwr/preview/mmwrhtml/mm6019a5.htm.
51. Koller K, Brown T, Spurgeon A, Levy L. Recent developments in low-level lead exposure and intellectual impairment in children. Environ Health Perspect. 2004;112:987–94.
52. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for lead. Atlanta: U.S. Department of Health and Human Services; 2019.
53. Gundacker C, Hengstschläger M. The role of the placenta in fetal exposure to heavy metals. WMW Wiener Medizinische. 2012;126:201–6.
54. Perkins M, Wright RO, Amarasiriwardena CJ, Jayawardene I, Rifas-Shiman SL, Oken E. Very low maternal lead level in pregnancy and birth outcomes in an eastern Massachusetts population. Ann Epidemiol. 2014;24:915–9.
55. Nishioka E, Yokoyama K, Matsukawa T, Vigeh M, Hirayama S, Ueno T, et al. Evidence that birth weight is decreased by maternal lead levels below 5mug/dl in male newborns. Reprod Toxicol. 2014;47:21–6.
56. Al Jameil N. Maternal serum lead levels and risk of preeclampsia in pregnant women: a cohort study in a maternity hospital, Riyadh, Saudi Arabia. Int J Clin Exp Pathol. 2014;7:3182–9.
57. Jeong KS, Park H, Ha E, Hong YC, Ha M, Park H, et al. Evidence that cognitive deficit in children is associated not only with iron deficiency, but also with blood lead concentration: a preliminary study. J Trace Elem Med Biol. 2015;29:336–41.
58. Taylor CM, Golding J, Emond AM. Adverse effects of maternal lead levels on birth outcomes in the ALSPAC study: a prospective birth cohort study. BJOG. 2015;122:322–8.
59. Taylor CM, Tilling K, Golding J, Emond AM. Low level lead exposure and pregnancy outcomes in an observational birth cohort study: dose-response relationships. BMC Res Notes. 2016;9:291.
60. Rodosthenous RS, Burris HH, Svensson K, Amarasiriwardena CJ, Cantoral A, Schnaas L, et al. Prenatal lead exposure and fetal growth: smaller infants have heightened susceptibility. Environ Int. 2017;99:228–33.
61. Renzetti S, Just AC, Burris HH, Oken E, Amarasiriwardena C, Svensson K, et al. The association of lead exposure during pregnancy and childhood anthropometry in the Mexican progress cohort. Environ Res. 2017;152:226–32.
62. Cassidy-Bushrow AE, Sitarik AR, Havstad S, Park SK, Bielak LF, Austin C, et al. Burden of higher lead exposure in African-Americans starts in utero and persists into childhood. Environ Int. 2017;108:221–7.

63. Hu J, Xia W, Pan X, Zheng T, Zhang B, Zhou A, Buka SL, Bassig BA, Liu W, Wu C, Peng Y, Li J, Zhang C, Liu H, Jiang M, Wang Y, Zhang J, Huang Z, Zheng D, Shi K, Qian Z, Li Y, Xu S. Association of adverse birth outcomes with prenatal exposure to vanadium: a population-based cohort study. Lancet Planet Health. 2017;1:e230–41.
64. Li J, Wang H, Hao JH, Chen YH, Liu L, Yu Z, et al. Maternal serum lead level during pregnancy is positively correlated with risk of preterm birth in a Chinese population. Environ Pollut. 2017;227:484–9.
65. Cheng L, Zhang B, Huo WQ, Cao ZQ, Liu WY, Liao JQ, et al. Fetal exposure to lead during pregnancy and the risk of preterm and early-term deliveries. Int J Hyg Environ Health. 2017;220:984–9.
66. Huang S, Xia W, Sheng X, Qiu L, Zhang B, Chen T, et al. Maternal lead exposure and premature rupture of membranes: a birth cohort study in China. BMJ Open. 2018;8:e021565.
67. Sanders AP, Svensson K, Gennings C, Burris HH, Oken E, Amarasiriwardena C, et al. Prenatal lead exposure modifies the effect of shorter gestation on increased blood pressure in children. Environ Int. 2018;120:464–71.
68. Parajuli RP, Fujiwara T, Umezaki M, Watanabe C. Impact of caste on the neurodevelopment of young children from birth to 36 months of age: a birth cohort study in Chitwan Valley, Nepal. BMC Pediatr. 2014;14:56.
69. Liu JA, Gao DG, Chen YM, Jing J, Hu QS, Chen YJ. Lead exposure at each stage of pregnancy and neurobehavioral development of neonates. Neurotoxicology. 2014;44:1–7.
70. Skerfving S, Lofmark L, Lundh T, Mikoczy Z, Stromberg U. Late effects of low blood lead concentrations in children on school performance and cognitive functions. Neurotoxicology. 2015;49:114–20.
71. Evens A, Hryhorczuk D, Lanphear BP, Rankin KM, Lewis DA, Forst L, et al. The impact of low-level lead toxicity on school performance among children in the Chicago Public Schools: a population-based retrospective cohort study. Environ Health. 2015;14:21.
72. Xu J, Hu H, Wright R, Sanchez BN, Schnaas L, Bellinger DC, et al. Prenatal lead exposure modifies the impact of maternal self-esteem on children's inattention behavior. J Pediatr. 2015;167:435–41.
73. Shah-Kulkarni S, Ha M, Kim BM, Kim E, Hong YC, Park H, et al. Neurodevelopment in early childhood affected by prenatal lead exposure and iron intake. Medicine. 2016;95:e2508.
74. Bhang SY, Ha E, Park H, Ha M, Hong YC, Kim BN, et al. Maternal stress and depressive symptoms and infant development at six months: the mothers and children's environmental health (MOCEH) prospective study. J Korean Med Sci. 2016;31:843–51.
75. Zhou L, Xu J, Zhang J, Yan C, Lin Y, Jia Y, et al. Prenatal maternal stress in relation to the effects of prenatal lead exposure on toddler cognitive development. Neurotoxicology. 2017;59:71–8.
76. Taylor CM, Kordas K, Golding J, Emond AM. Data relating to prenatal lead exposure and child IQ at 4 and 8 years old in the Avon Longitudinal Study of Parents and Children. Neurotoxicology. 2017;62:224–30.
77. Winter AS, Sampson RJ. From lead exposure in early childhood to adolescent health: a Chicago birth cohort. Am J Public Health. 2017;107:1496–501.
78. Reuben A, Caspi A, Belsky DW, Broadbent J, Harrington H, Sugden K, et al. Association of childhood blood lead levels with cognitive function and socioeconomic status at age 38 years and with IQ change and socioeconomic mobility between childhood and adulthood. JAMA. 2017;317:1244–51.
79. Ji YL, Hong XM, Wang GY, Chatterjee N, Riley AW, Lee LC, et al. A prospective birth cohort study on early childhood lead levels and attention deficit hyperactivity disorder: new insight on sex differences. J Pediatr. 2018;199:124–31.
80. Nye MD, King KE, Darrah TH, Maguire R, Jima DD, Huang ZQ, et al. Maternal blood lead concentrations, DNA methylation of MEG3 DMR regulating the DLK1/MEG3 imprinted domain and early growth in a multiethnic cohort. Environ Epigenet. 2016;2:dvv009.

81. Sergeyev O, Burns JS, Williams PL, Korrick SA, Lee MM, Revich B, et al. The association of peripubertal serum concentrations of organochlorine chemicals and blood lead with growth and pubertal development in a longitudinal cohort of boys: a review of published results from the Russian Children's Study. Rev Environ Health. 2017;32:83–92.
82. Camaj PR, Graziano JH, Preteni E, Popovac D, LoIacono N, Balac O, et al. Long-term effects of environmental lead exposure on blood pressure and plasma soluble cell adhesion molecules in young adults: A follow-up study of a prospective cohort in Kosovo. J Environ Public Health. 2018;2018:3180487.
83. Farzan SF, Howe CG, Chen Y, Gilbert-Diamond D, Cottingham KL, Jackson BP, et al. Prenatal lead exposure and elevated blood pressure in children. Environ Int. 2018;121:1289–96.
84. Joo HCJ, Burm E, Park H, Hong YC, Kim Y, Ha EH, Kim Y, Kim BN, Ha M. Gender difference in the effects of lead exposure at different time windows on neurobehavioral development in 5-year-old children. Sci Total Environ. 2018;615:1086–92.
85. Faulk C, Liu K, Barks A, et al. Longitudinal epigenetic drift in mice perinatally exposed to lead. Epigenetics. 2014;9(7):934–41.
86. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for manganese. Atlanta: U.S. Department of Health and Human Services; 2012.
87. Bouchard M, Laforest F, Vandelac L, Bellinger D, Mergler D. Air manganese and hyperactive behaviors: pilot study of school-age children exposed through tap water. Environ Health Perspect. 2007;115(1):122–7.
88. Lucchini R, Guazzetti S, Zoni S, Donna F, Peter S, Zacco A, et al. Tremor, olfactory and motor changes in Italian adolescents exposed to historical ferro-manganese emission. Neurotoxicology. 2012;33:687–96.
89. Aschner M, Guilarte TR, Schneider JS, Zheng W. Manganese: recent advances in understanding its transport and neurotoxicity. Toxicol Appl Pharmacol. 2007;221(2):131–47.
90. Erikson KM, Thompson K, Aschner J, Aschner M. Manganese neurotoxicity: a focus on the neonate. Pharmacol Ther. 2007;113:369–77.
91. Vigeh M, Yokoyama K, Ohtani K, Shahbazi F, Matsukawa T. Increase in blood manganese induces gestational hypertension during pregnancy. Hypertens Pregnancy. 2013;32:214–24.
92. Yu X, Cao L, Yu X. Elevated cord serum manganese level is associated with a neonatal high ponderal index. Environ Res. 2013;121:79–83.
93. Eum JH, Cheong HK, Ha EH, Ha M, Kim Y, Hong YC, et al. Maternal blood manganese level and birth weight: a MOCEH birth cohort study. Environ Health. 2014;13:31.
94. Chen L, Ding G, Gao Y, Wang P, Shi R, Huang H, et al. Manganese concentrations in maternal-infant blood and birth weight. Environ Sci Pollut Res Int. 2014;21:6170–5.
95. Guan H, Wang M, Li X, Piao F, Li Q, Xu L, et al. Manganese concentrations in maternal and umbilical cord blood: related to birth size and environmental factors. Eur J Public Health. 2014;24:150–7.
96. Mora AM, van Wendel de Joode B, Mergler D, Cordoba L, Cano C, Quesada R, et al. Blood and hair manganese concentrations in pregnant women from the infants' environmental health study (ISA) in Costa Rica. Environ Sci Technol. 2014;48:3467–76.
97. Tsai MS, Liao KW, Chang CH, Chien LC, Mao IF, Tsai YA, et al. The critical fetal stage for maternal manganese exposure. Environ Res. 2015;137:215–21.
98. Rahman SM, Kippler M, Ahmed S, Palm B, El Arifeen S, Vahter M. Manganese exposure through drinking water during pregnancy and size at birth: a prospective cohort study. Reprod Toxicol. 2015;53:68–74.
99. Mora AM, Arora M, Harley KG, Kogut K, Parra K, Hernandez-Bonilla D, et al. Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. Environ Int. 2015;84:39–54.
100. Bakouei S, Reisian F, Lamyian M, Haji Zadeh E, Zamanian H, Taheri Kharameh Z. High intake of manganese during second trimester, increases the risk of preterm delivery: a large scale cohort study. Glob J Health Sci. 2015;7:226–32.

101. Xia W, Du X, Zheng T, Zhang B, Li Y, Bassig BA, et al. A case-control study of prenatal thallium exposure and low birth weight in China. Environ Health Perspect. 2016;124:164–9.
102. Arbuckle TE, Liang CL, Morisset AS, Fisher M, Weiler H, Cirtiu CM, et al. Maternal and fetal exposure to cadmium, lead, manganese and mercury: the MIREC study. Chemosphere. 2016;163:270–82.
103. Ashley-Martin J, Dodds L, Arbuckle TE, Ettinger AS, Shapiro GD, Fisher M, et al. Maternal and cord blood manganese (Mn) levels and birth weight: the MIREC birth cohort study. Int J Hyg Environ Health. 2018;221:876–82.
104. Lee JJ, Valeri L, Kapur K, Ibne Hasan MOS, Quamruzzaman Q, Wright RO, et al. Growth parameters at birth mediate the relationship between prenatal manganese exposure and cognitive test scores among a cohort of 2- to 3-year-old Bangladeshi children. Int J Epidemiol. 2018;47:1169–79.
105. Tsuji M, Shibata E, Morokuma S, Tanaka R, Senju A, Araki S, et al. The association between whole blood concentrations of heavy metals in pregnant women and premature births: the Japan Environment and Children's Study (JECS). Environ Res. 2018;166:562–9.
106. Lewis RC, Meeker JD, Basu N, Gauthier AM, Cantoral A, Mercado-Garcia A, et al. Urinary metal concentrations among mothers and children in a Mexico City birth cohort study. Int J Hyg Environ Health. 2018;221:609–15.
107. Claus Henn B, Ettinger AS, Schwartz J, Tellez-Rojo MM, Lamadrid-Figueroa H, Hernandez-Avila M, et al. Early postnatal blood manganese levels and children's neurodevelopment. Epidemiology. 2010;21:433–9.
108. Lin CC, Chen YC, Su FC, Lin CM, Liao HF, Hwang YH, et al. In utero exposure to environmental lead and manganese and neurodevelopment at 2 years of age. Environ Res. 2013;123:52–7.
109. Yu X, Chen L, Wang C, Yang X, Gao Y, Tian Y. The role of cord blood BDNF in infant cognitive impairment induced by low-level prenatal manganese exposure: LW birth cohort, china. Chemosphere. 2016;163:446–51.
110. Langley RL, Kao Y, Mort SA, Bateman A, Simpson BD, Reich BJ. Adverse neurodevelopmental effects and hearing loss in children associated with manganese in well water, North Carolina, USA. J Environ Occup Sci. 2015;4:62–9.
111. Ode A, Rylander L, Gustafsson P, Lundh T, Kallen K, Olofsson P, et al. Manganese and selenium concentrations in umbilical cord serum and attention deficit hyperactivity disorder in childhood. Environ Res. 2015;137:373–81.
112. Gunier RB, Arora M, Jerrett M, Bradman A, Harley KG, Mora AM, et al. Manganese in teeth and neurodevelopment in young Mexican-American children. Environ Res. 2015;142:688–95.
113. Chung SE, Cheong HK, Ha EH, Kim BN, Ha M, Kim Y, et al. Maternal blood manganese and early neurodevelopment: the mothers and children's environmental health (MOCEH) study. Environ Health Perspect. 2015;123:717–22.
114. Mora AM, van Wendel de Joode B, Mergler D, Cordoba L, Cano C, Quesada R, et al. Maternal blood and hair manganese concentrations, fetal growth, and length of gestation in the Isa cohort in Costa Rica. Environ Res. 2015;136:47–56.
115. Yu XD, Zhang J, Yan CH, Shen XM. Prenatal exposure to manganese at environment relevant level and neonatal neurobehavioral development. Environ Res. 2014;133:232–8.
116. Rodrigues EG, Bellinger DC, Valeri L, Hasan MO, Quamruzzaman Q, Golam M, et al. Neurodevelopmental outcomes among 2- to 3-year-old children in Bangladesh with elevated blood lead and exposure to arsenic and manganese in drinking water. Environ Health. 2016;15:44.
117. Rahman SM, Kippler M, Tofail F, Bolte S, Hamadani JD, Vahter M. Manganese in drinking water and cognitive abilities and behavior at 10 years of age: a prospective cohort study. Environ Health Perspect. 2017;125:057003.
118. Claus Henn B, Bellinger DC, Hopkins MR, Coull BA, Ettinger AS, Jim R, et al. Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted superfund site. Environ Health Perspect. 2017;125:067020.

119. Valeri L, Mazumdar MM, Bobb JF, Claus Henn B, Rodrigues E, Sharif OIA, et al. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20-40 months of age: evidence from rural Bangladesh. Environ Health Perspect. 2017;125:067015.
120. Haynes EN, Sucharew H, Hilbert TJ, Kuhnell P, Spencer A, Newman NC, et al. Impact of air manganese on child neurodevelopment in East Liverpool, Ohio. Neurotoxicology. 2018;64:94–102.
121. Freire C, Amaya E, Gil F, Fernandez MF, Murcia M, Llop S, et al. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: the Environment and Childhood (INMA) project. Sci Total Environ. 2018;621:340–51.
122. Munoz-Rocha TV, Tamayo YOM, Romero M, Pantic I, Schnaas L, Bellinger D, et al. Prenatal co-exposure to manganese and depression and 24-months neurodevelopment. Neurotoxicology. 2018;64:134–41.
123. Mora AM, Cordoba L, Cano JC, Hernandez-Bonilla D, Pardo L, Schnaas L, et al. Prenatal mancozeb exposure, excess manganese, and neurodevelopment at 1 year of age in the infants' environmental health (ISA) study. Environ Health Perspect. 2018;126:057007.
124. Lucchini R, Placidi D, Cagna G, Fedrighi C, Oppini M, Peli M, Zoni S. Manganese and developmental neurotoxicity. Adv Neurobiol. 2017;18:13–34.
125. Li Y, Wu J, Zhou W, Gao E. Effects of manganese on routine semen quality parameters: results from a population-based study in China. BMC Public Health. 2012;12:919.
126. Ebisu K, Bell ML. Airborne PM2·5 chemical components and low birth weight in the northeastern and mid-Atlantic regions of the United States. Environ Health Perspect. 2012;120:1746–52.
127. Morgan AM, El-Tawil OS. Effects of ammonium metavanadate on fertility and reproductive performance of adult male and female rats. Pharmacol Res. 2003;47:75–85.
128. Jiang MM, Li YY, Zhang B, Zhou AF, Zheng TZ, Qian ZM, et al. A nested case-control study of prenatal vanadium exposure and low birthweight. Hum Reprod. 2016;31:2135–41.
129. Jin S, Xia W, Jiang Y, Sun X, Huang S, Zhang B, Zhou A, Zheng T, Xu S, Li Y. Urinary vanadium concentration in relation to premature rupture of membranes: a birth cohort study. Chemosphere. 2018;210:1035–41.
130. Hu J, Peng Y, Zheng T, Zhang B, Liu W, Wu C, Jiang M, Braun JM, Liu S, Buka SL, Zhou A, Wise JP, Zhang Y, Jiang Y, Hu C, Chen X, Huang Z, Zheng D, Shi K, Zhang X, Truong A, Qian Z, Xia W, Li Y, Xu S. Effects of trimester-specific exposure to vanadium on ultrasound measures of fetal growth and birth size: a longitudinal prospective prenatal cohort study. Lancet Planet Health. 2018;2:427–37.
131. Jiang Y, Xia W, Zhang B, Pan X, Liu W, Jin S, Huo W, Liu H, Peng Y, Sun X, Zhang H, Zhou A, Xu S, Li Y. Predictors of thallium exposure and its relation with preterm birth. Environ Pollut. 2018;233:971–6. https://doi.org/10.1016/j.envpol.2017.09.080.
132. Qi J, Liang CM, Yan SQ, Li ZJ, Li J, Huang K, Xiang HY, Tao YR, Hao JH, Tong SL, Tao FB. Study on the relationship of thallium exposure and outcomes of births. Zhonghua Liu Xing Bing Xue Za Zhi. 2018;39(8):1112–6. https://doi.org/10.3760/cma.j.issn.0254-6450.2018.08.019.
133. Qi J, Lai Y, Liang C, Yan S, Huang K, Pan W, et al. Prenatal thallium exposure and poor growth in early childhood: a prospective birth cohort study. Environ Int. 2019;123:224–30.
134. Rodríguez-Mercado JJ, Altamirano-Lozano MA. Genetic toxicology of thallium: a review. Drug Chem Toxicol. 2013;36:369–83.
135. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological profile for thallium. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 1992.
136. Peter AL, Viraraghavan T. Thallium: a review of public health and environmental concerns. Environ Int. 2005;31:493–501.
137. Hoffman RS. Thallium poisoning during pregnancy: a case report and comprehensive literature review. J Toxicol Clin Toxicol. 2000;38:767–75.

Chapter 12
Environmental Pollution and Recent Data on Asian Children's Health in Relation to Pre- and Early Post-natal Exposure to Persistent Organic Pollutants, Including PCBs, PCDD/PCDFs, and Organochlorine Pesticides

Chihiro Miyashita

Abstract Chemical compounds containing persistent organic pollutants (POPs) have been banned or restricted from production and use since the 1970s, and in more recent times by the Stockholm Convention. This is because past incidence of poisonings from accidental exposure to high levels of POPs, as well as findings from animal experiments, has led to serious health concerns. POPs have lipophilicity and long half-life in the human body, and contain potential endocrine disruptors, including polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, dibenzofurans, and organochlorine pesticides. Even today, POPs continue to exist and bio-accumulate in the environment, the food chain, and the human body because these chemicals resist biodegradation. POPs have been widely detected in soil, wild animals, and bodies of human beings living throughout the world. Recent studies report that environmental pollution from electronic-waste processing in certain areas of Asian countries may be associated with impaired health in children. In addition, even adults who live in non-industrial areas and have no history of accidental poisoning have been chronically exposed to low levels of POPs in their daily life through food consumption, use of products that contain POPs, and absorption from the surrounding environment. In particular, the fetal development and early post-natal periods may be critical windows when individuals are most susceptible to the toxic effects of POPs. Many studies that include data from incidents of acute exposure to high levels of POPs in humans have been reported; however, limited information is available on exposure to low levels of POPs in the general population. It is especially necessary to clarify long-term effects of chronic exposure to low levels of POPs among vulnerable populations that include developing fetuses, infants, and children, through well-designed

C. Miyashita (✉)
Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan
e-mail: miyasita@med.hokudai.ac.jp

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_12

human epidemiological studies. This chapter describes the effects of prenatal and early post-natal exposure to low levels of POPs on children's health in general populations, focusing on Asian countries, where different exposure profiles and lifestyle factors exist compared to Western countries and other parts of the world.

Keywords Persistent organic pollutants (POPs) · Polychlorinated biphenyl (PCBs) · DLC · Dioxin-like compound (DLCs) · Organochlorine pesticides (OCPs) · Pre-natal and early post-natal exposure · Children's health · Asian countries

Abbreviations

AHR	Aromatic hydrocarbon receptor
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DHEA	Dehydroepiandrosterone
DLC	Dioxin-like compound
e-waste	Electronic waste
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HOMA-β	The homeostatic model assessment for β-cell function
Ig	Immunoglobulin
OCPs	Organochlorine pesticides
p,p′-DDD	1,1-dichloro-2, 2-bis(4-chlorophenyl)ethane
p,p′-DDT	1,1,1-trichloro-2,2-bis-(p-chlorophenyl)-ethane
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxins
PCDF	Polychlorinated dibenzofuran
POPs	Persistent organic pollutants
TCDD	Tetrachlorodibenzo-p-dioxin
TEF	Toxic equivalency factor
TEQ	Toxic equivalent
WHO	World Health Organization
β-BHC	Beta-hexachlorocyclohexane

12.1 Introduction to POPs and Exposure in Utero and in Early Post-natal Period

Persistent organic pollutants (POPs) targeted by the Stockholm Convention (http://chm.pops.int) are chemicals in the environment that cause global concern due to their potential for long-range transport, their persistence in the environment, their

ability to bio-magnify and bio-accumulate in ecosystems, and their significant negative effects on human health and the environment. (http://www.who.int/foodsafety/areas_work/chemical-risks/pops/en/). The human fetus in utero is supplied with various substances of external origin from the mother's circulation via the placenta and umbilical cord. These substances include thyroid and steroid hormones, lipids and nutrients, and serve to regulate normal pregnancy and fetal development. The developmental stages of fetus, newborn, and infant are most susceptible to the external environment, due to immature organs and tissues and reduced detoxification ability. Exposure to POPs in utero causes serious health concerns because POPs imitate the structure and action of these substances of external origin and can cross placental and blood–brain barriers to reach immature fetal organs and tissues. After birth, newborns and infants experience lactational exposure to POPs via maternal breast milk, which may contain lipids contaminated with high levels of POPs. Prenatal and lactational exposure to POPs can chronically influence the health of children throughout their lives (Fig. 12.1). POPs consist of three different types. The first type includes industrial chemicals that have been artificially synthesized. The second type comprises unintended by-products that are unexpectedly generated. The third type are pesticides used for vector control in some malaria endemic areas (Figs. 12.2 and 12.3). Industrial chemicals and unintended by-products are associated with industrialization and urbanization, on the other hand, pesticides are associated with vector control. So, distributions observed in the use of industrial chemicals and unintended by-products vs. pesticides differ among countries and regions [1].

This chapter focuses on the POPs consisting of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and organochlorine pesticides (OCPs). PCBs, PCDDs, PCDFs, and OCPs have a long half-life (over 5–20 years) of elimination from the human body and may accumulate in bodies of women who will bear children. They thus can be transferred from a pregnant woman to her developing fetus and will accumulate for a long time in the body of the children who are born to these mothers [2]. PCBs have 209 congeners, which are divided into two categories, non-dioxin-like PCBs and dioxin-like PCBs (Table 12.1). Congener PCB-153 is one of the non-dioxin-like PCBs detected with the largest contribution to total PCBs in humans and used as an indicator of exposure level of PCBs. Therefore, PCB 153 is useful for comparisons of exposure to PCBs among different populations. Potential toxic effects of non-dioxin-like PCBs are considered to be lower than that of dioxin-like PCBs because of their weakened affinity for aryl hydrocarbon receptors (AHR). Dioxin-like-PCBs have 12 congeners, which are divided into 2 groups, non-ortho PCBs and mono-ortho PCBs. POPs of unintended by-products have 17 congeners, which are also divided into two groups, 7 PCDDs and 10 PCDFs. The 17 PCDDs/PCDFs and 12 dioxin-like PCBs are known as dioxin-like compounds (DLCs) (Table 12.2). Recently, the term *dioxin* has been used as meaning for DLCs. Potential toxic effects of DLCs are related to AHR binding affinity. A World Health Organization (WHO) panel has assigned a toxic equivalency factor (TEF) to each of the 29 DLCs based on an estimate of AHR binding affinity. TEFs are used to identify the toxic effects of the 29 DLCs based on their relationship to the most toxic, 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD).

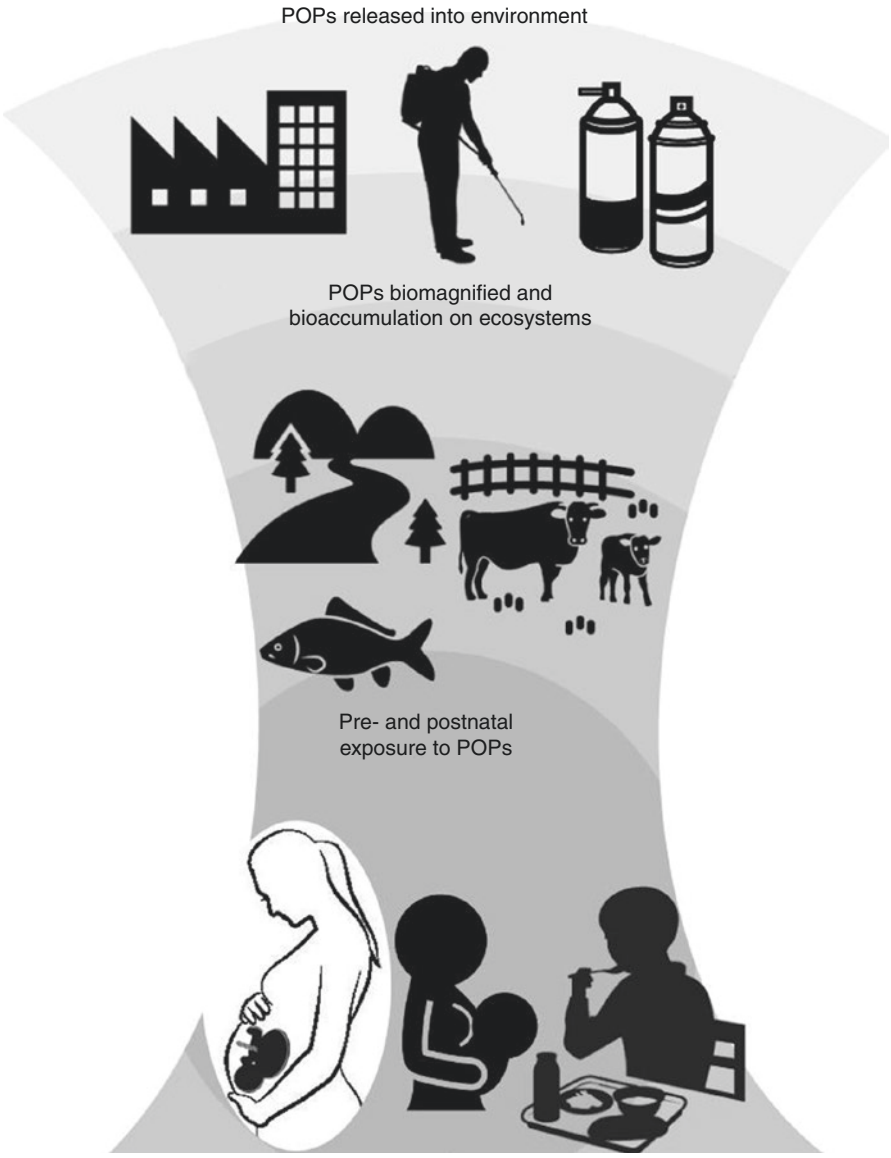


Fig. 12.1 The pathways of pre- and postnatal exposure to Persistent organic pollutants

TCDDs are assigned a basic coefficient value of 1 TEF, and the other 28 DLCs are each assigned a coefficient value ranging downward from 1 to 0.00003. The Toxic Equivalent Quantity (TEQ) is calculated by multiplying the concentration of the individual congener of DLC by its TEF [3]. Pesticides that are POPs include organochlorine pesticides (OCPs), such as aldrin, chlordane, dieldrin, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene (HCB), mirex, dichlorodiphenyltrichloroethane (DDT), toxaphene, and hexachlorocyclohexanes (HCH). These were used as

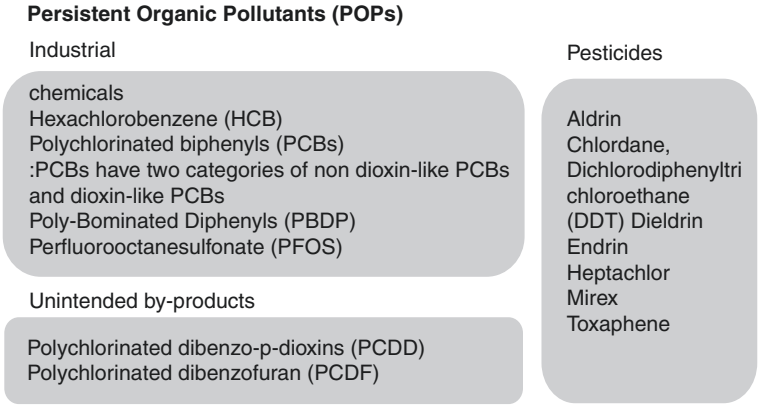


Fig. 12.2 Categories of Persistent organic pollutants

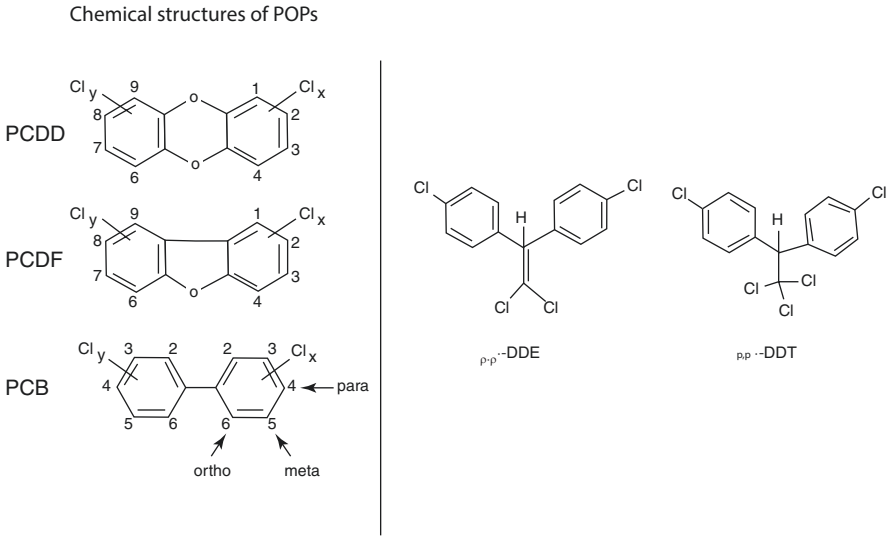


Fig. 12.3 Chemical structures of Persistent organic pollutants

Table 12.1 Non-dioxin-like PCBs and dioxin-like PCBs

	Non-dioxin-like PCBs	Dioxin-like PCBs
PCB congener type	Other PCBs exuding 12 dioxin-like PCBs	12 PCBs include 4 non-ortho PCBs and 8 mono-ortho PCBs
Affinity to AHR	Low	High
TEF	No	0.01–0.00001
Potential toxic effect	Low	High

TEF toxic equivalency factor, *PCB* polychlorobiphenyl

Table 12.2 Dioxin-like compounds (DLCs) and TEF

Congener	WHO 2005 TEF
17 congeners of PCDDs/PCDFs (TEQ pg/g lipid)	
PCDDs (polychlorinated dibenzo-p-dioxins)	
2,3,7,8-TCDD	1.0
1,2,3,7,8-PeCDD	1.0
1,2,3,4,7,8-HxCDD	0.1
1,2,3,6,7,8-HxCDD	0.1
1,2,3,7,8,9-HxCDD	0.1
1,2,3,4,6,7,8-HpCDD	0.0
OCDD	0.0
PCDFs (polychlorinated dibenzofurans)	
2,3,7,8-TCDF	0.1
1,2,3,7,8-PeCDF	0.0
2,3,4,7,8-PeCDF	0.3
1,2,3,4,7,8-HxCDF	0.1
1,2,3,6,7,8-HxCDF	0.1
2,3,4,6,7,8-HxCDF	0.1
1,2,3,7,8,9-HxCDF	0.1
1,2,3,4,6,7,8-HpCDF	0.010
1,2,3,4,7,8,9-HpCDF	0.010
OCDF	0.0001
12 congeners of dioxin-like PCBs (TEQ pg/g lipid)	
Non-ortho–PCBs	
344′5-TCB (PCB 81)	0.0001
33′44′-TCB (PCB 77)	0.0001
33′44′5-PenCB (PCB 126)	0.1
33′44′55′-HxCB (PCB 169)	0.03000
Mono-ortho–PCBs	
2′344′5-PeCB (PCB 123)	0.00003
23′44′5-PeCB (PCB 118)	0.00003
2344′5-PeCB (PCB 114)	0.00003
233′44′-PeCB (PCB 105)	0.00003
23′44′55′-HxCB (PCB 167)	0.00003
233′44′5-HxCB (PCB 156)	0.00003
233′44′5′-HxCB (PCB 157)	0.00003
233′44′55′-HpCB (PCB 189)	0.00003

TEF toxic equivalency factor, *TEQ* toxic equivalent, *PCB* polychlorobiphenyl [3]

agricultural pesticides throughout the world until the 1980s, and are now prohibited in many countries. However, organochlorine pesticides and their metabolites, including p,p′-dichlorodiphenyldichloroethylene (DDE), d-HCH, hexachlorobenzene, 1, 1, 1-trichloro-2, 2-bis-(p-chlorophenyl)-ethane (p,p′-DDT), 1,1-dichloro-2, 2-bis(4-chlorophenyl)ethane (p,p′-DDD), beta-hexachlorocyclohexane (β-BHC), oxychlordane, and trans-nonachlor, are still detected in the environment and human bodies [4].

12.2 Toxic Mechanisms Involved in the Effects of POPs on children's Health

The mechanisms of POPs-induced developmental toxicity are not completely understood; however, studies suggest that exposure to POPs may induce endocrine-disruption, activate nuclear receptors, and change signaling pathways in animals and humans. Experimental studies have demonstrated the endocrine-disrupting effects of PCBs through their ability to stimulate estrogen and their ability to function as xenoestrogens [5]. DLCs induce toxicity by binding to AHR, which plays an important role in a variety of cellular processes, including neuronal development, cell fate determination, and cell differentiation. DLCs bind to AHRs and disrupt normal fetal development [3]. Moreover, estrogen receptors and AHR are ligand-activated transcription factors, and estrogen receptors and AHR signaling pathways interact with each other. The activity of estradiol is mediated by the estrogen receptor (ER), and production and inhibition of multiple steroid hormones, including estradiol, testosterone, and dehydroepiandrosterone (DHEA), as well as reproductive hormones such as inhibin B, are regulated. Therefore, it is thought that DLCs may cause disturbances of steroid and reproductive hormones in animals and humans.

The effects on human steroid hormones from exposure to POPs in utero include an increase in maternal DLCs associated with decreased testosterone/estradiol ratios and inhibin B levels, and increased DHEA levels and adrenal androgen/glucocorticoid ratios in male cord blood. Exposure to DLCs in utero may inhibit fetal reproductive development and steroid hormone synthesis [6]. The effects of exposure to POPs in utero on the retardment of neurodevelopment results from fetal brain tissue expression of thyroid hormone receptors and the fetus being completely dependent on maternal supply of thyroxine during the first and second trimesters of pregnancy. Some PCBs are similar in structure to thyroid hormones and interact with thyroid hormone-binding proteins and thyroid hormone receptors, which may directly affect thyroid function. DLCs may indirectly affect thyroid function by disturbing thyroid hormone-binding proteins. POPs exposure in utero may affect neuronal connectivity in hippocampal and hypothalamic neurons, and impair cognitive function and neurobehavioral conditions. Suspected adverse effects of exposure to POPs have been reported for attention deficit hyperactivity disorder and autism spectrum disorders [7]. In human studies, retardment of neurodevelopment was observed in children at 6, 18, and 24 months of age in line with increasing POPs levels in maternal blood during pregnancy, even at low levels [8, 9]. However, in a study completed among 11-year-old children in Hong Kong, no association was observed between neurocognitive function, as measured with psychological tests, and maternal dioxin levels measured in breast milk [10]. Further study is needed that includes follow-up of subjects in whom adverse effects of exposure to POPs in utero were observed, in order to see if the effects remain or disappear during adolescence.

Regarding the effects of exposure to POPs in utero on immunotoxicity, animal studies have demonstrated that fetal TCDD exposure inhibits cellular differentiation and maturation, particularly in T lymphocytes, causes thymic atrophy, and leads to

immunosuppression in offspring [4]. At comparable environmental levels, exposure to complex mixtures of DLCs may induce immunosuppression in both mice and humans [11]. In a study done in Japan, DLC levels found in breast milk were significantly associated with an increase in the lymphocyte subset ratio in peripheral blood of breastfed infants at 10 months of age [12]. A case–control study of asthma in preschool children carried out in Kumamoto, Japan, found a significant correlation between interleukin-8 and the serum levels of individual PCB congeners in asthmatic children [13]. The Inuit people have traditionally consumed marine mammals, which exposes them to high levels of POPs. A study of the Inuit people living on Faeroe Island suggests that exposure to PCBs in utero may reduce vaccine antibody reactions in children for as long as 7 years after birth [14]. Moreover, the study of Inuit people reported that both the prevalence of infectious disease, which included acute otitis media in children, and the incidence of respiratory disorders after birth were increased with exposure level to PCBs [4].

Metabolic syndrome is a cluster of metabolic abnormalities that includes obesity, glycosemia, reduced high-density lipoprotein cholesterol levels, hypertriglyceridemia, and hypertension. Possible toxic mechanisms of POPs are suspected to induce inflammation response and disrupt endocrine metabolism and adipocyte function. Human studies in adults have shown that POPs levels are associated with a risk of reproductive problems and metabolic disease. Previous experiments and epidemiological studies suggest that pre- and post-natal exposure to POPs may be associated with increased risk of metabolic dysfunction such as obesity, insulin resistance, dyslipidemia, hypertension in infancy and adolescence [15].

12.3 Accidental High-Level Poisoning in Specific Populations

Since the 1960s, concerns about chemical pollution in the global ecosystem have been raised. This is because production and use of synthetic chemicals have increased with rapid industrialization and urbanization. Along with industrial progress, workers and residents in high pollution areas in and near manufacturing plants and industrial waste facilities have been exposed to high levels of PCBs and DLCs. DLCs have been known to be emitted from waste incinerators that have a poor system of combustion control. In the Seveso accident of 1976, TCDD was unintentionally released from a chemical plant near the town of Seveso, Italy, and the surrounding environment, and people living there were exposed to high levels of contamination. After the accident occurred, the sex distribution at birth of children who were born from fathers exposed to high TCDD (serum TCDD concentrations of greater than 15 ppt) and younger than 19 years old in 1976 was a change to an increased proportion of female births [16]. In another case going back to the 1960s, pregnant women living in the San Francisco Bay Area had a median of 1.1 μg/L serum level of PCB 153, and a lower male birth ratio was observed with increasing concentrations of this POPs [17]. These reports from the Seveso accident and the San Francisco Bay Area demonstrate that prenatal exposure to high PCBs and DLCs can induce adverse effects on the survival of male fetuses.

In the 1960s, specific regions in Vietnam were highly contaminated by the most toxic form of TCDD which is found in the herbicide Agent Orange. This substance had been sprayed into the environment during the Vietnam War. The regions contaminated by TCDD are called hotspots. Agent Orange is known to affect reproduction and has been observed to alter levels of steroid hormones in laboratory rodents and other species. A recent study found an inverse correlation between serum levels of TCDD and testosterone in US veterans who were stationed in Vietnam during the war. Several epidemiologic studies have prospectively assessed the association between circulating concentrations of sex steroid hormones and prostate cancer. High levels of TCDD detected in maternal breast milk in Vietnamese mothers who lived in hotspots were 1832 pg/g lipid. PCDD/PCDFs congeners in maternal breast milk inversely correlated with DHEA levels and salivary adrenal androgen/glucocorticoid ratios in 3-year-old children born to these mothers [18]. Another study conducted in Vietnam suggests the possibility that decreased cord blood testosterone levels related to prenatal exposure to PCDD/PCDFs is more pronounced in females [19]. In the previous study that targeted mother/infant pairs who were living in the Vietnamese hotspot, the Bayley Scales of Infant and Toddler Development-III were used to assess developmental functioning in children. The tests measure development across 5 scales: cognitive, receptive, and expressive language, and fine and gross motor skills, as well as additional measures of social-emotional and adaptive behavior. No significant association was observed between DLCs in breast milk and neurodevelopmental scores of cognitive, language, or motor function. However, decreased social–emotional scores were associated with increased DLC levels in maternal milk. These results suggest that perinatal exposure to DLCs may affect social–emotional development in 1-year-olds, without diminishing all neurodevelopmental functions [20]. In a follow-up investigation to this Vietnam study, motor function and expressive communication scores among boys were found to decrease during the first 3 years of life with increases in TCDD found in maternal breast milk. All body size measurements in boys were observed to be decreased in the high-exposure groups of 2,3,7,8-tetraCDD and PCDDs/PCDFs-TEQ. This study shows that perinatal DLC exposure affects physical growth and neurodevelopment in children throughout the first 3 years of life in a sex-specific manner [21] (Table 12.3).

A mass outbreak of poisoning occurred in western Japan in 1968 due to consumption of rice-bran oil contaminated with DLCs. The incident was called Yusho in Japanese. High levels of DLCs detected in blood among Yusho patients were 215.4 pg/g TEQ lipid [22]. PCDFs may be primarily responsible for the various Yusho symptoms observed. A similar mass outbreak of poisoning occurred in Taiwan in 1978–1979 due to the consumption of rice-bran oil contaminated with DLCs and polychlorinated quaterphenyls. This incident is called YuCheng (oil disease) in Taiwan. Detected high levels of PCDD/PCDFs in blood among YuCheng patients were 6550 pg/g TEQ lipid. Yusho and YuCheng patients were probably exposed to 10–30-fold higher DLCs compared with that of unexposed group. Babies born or taking breast milk from mothers of Yusho or YuCheng patients were called Yusho or YuCheng babies. Yusho babies had pigmentation of skin and gingivae; however, these symptoms generally improved within 3 months after birth [23].

Table 12.3 Recent epidemiological study evaluated association between dioxins and children's health in Vietnam

Author	Publication year	Country/ region	Study design	Number of subjects	Exposure	Outcomes	Main findings
Boda	2018	Bien Hoa city, South Vietnam	Cross-sectional study	162 mother-and-newborn pairs	Dioxins in breast milk	Sex hormone in cord blood	Higher TEQ-PCDD/Fs were associated with decreased testosterone level in girls
Kido	2016	Bien Hoa city, South Vietnam	Cohort study	104 mother-child pairs	Dioxins in breast milk	Dehydroepiandrosterone (DHEA) in salivary of children at 3-year-old age	Higher chlorinated dioxin congeners were associated with DHEA levels

Yusho babies also were found to have significant inverse associations between birth weight and PCDD, PCDF, and non-ortho PCB levels among male, but not female, infants [24]. Fourteen years after the YuCheng accident, the children had high PCDF levels of 89 pg/g lipid [25]. YuCheng children who were exposed in utero or during lactation to PCDD/PCDF and PCBs had higher incidence of bronchitis, reduced serum levels of immunoglobulin (Ig) A, IgG, and IgM at 6 months [26], and a higher incidence of influenza and otitis media at 6 years of age than a group of unexposed controls [27]. Moreover, developmental abnormalities, including lower body weight, shorter height, poorer cognitive development, lower IQ scores, and delayed sexual development were also observed in YuCheng children [23] (Table 12.4). These adverse effects were thought to be caused by the estrogenic or antiandrogenic properties and affinity of binding to AHR of DLCs in Yusho and YuCheng children.

Table 12.4 Human study of PCB/dioxins and children's health respect to Yusho and Yucheng accidents

Author	Publication year	Country/ region	Accidental poisoning (outbreak year)	Exposure	Main findings of Yusho/ Yucheng children
Tsukimori	2012	Japan	Yusho (1968)	Mother of Yusho patients who intake rice oil contaminated by PCDF/PCB	Significant inverse associations were found association between birth weight were also found for total PCDD TEQ, total PCDF TEQ, and total non-ortho PCB TEQ levels among male, but not female, infants
Guo	2004	Taiwan	Yucheng (1978–1979)	Mother of Yucheng patients who intake rice oil contaminated by PCDF/PCB	Lower body weight and height, poorer cognitive development, lower IQ scores, and delayed sexual development
Aoke	2001	Japan	Yusho (1968)	Mother of Yusho patients who intake rice oil contaminated by PCDF/PCB	Pigmentation of skin and gingivae.Adverse effects of physical and mental activities with Yusho children has not been clearly detected.
Yu	1998	Taiwan	Yucheng (1978–1979)	Mother of Yucheng patients who intake rice oil contaminated by PCDF/PCB	Higher frequencies of bronchitis, respiratory tract and ear infection
Chao	1997	Taiwan	Yucheng (1978–1979)	Mother of Yucheng patients who intake rice oil contaminated by PCDF/PCB	Higher incidence of middle-ear diseases

12.4 Environmental Pollution in Select Asian Countries

Rapid growth in production of electronic products has resulted in the problem of electronic-waste (e-waste) processing. Monitoring surveys recently reported that serious environmental pollution near electronic-waste processing sites in Asian countries such as India and China result from inappropriate discharge of PCBs and PCDD/PCDFs [28]. This has raised concerns about high exposure to these chemicals in residents and workers, and especially pregnant women and children who live in the area surrounding e-waste processing sites. At e-waste recycling sites in Eastern China, concentrations of total PCBs (sum of 33 PCB congeners) in pooled food samples collected from e-waste areas resulted in estimated exposures for children (2340 ng/kg bw/day) that substantially exceed the Minimal Risk Levels for the total PCBs of 20 ng/kg bw/day published in the Agency for Toxic Substances & Disease Registry. Moreover, concentrations of the sum of TEQ levels of 4 dioxin-like PCBs (PCB-105, 118, 156, and 167) resulted in dietary exposures for children (10.2 pg TEQ/kg bw/day) that exceed the WHO tolerable daily intake of 1–4 pg TEQ/kg bw/day [29].

In a cross-sectional study, school-age children living in an e-waste dismantling area in China had higher levels of PCDDs/PCDFs and thyroid hormones, such as free tri-iodothyronine (FT3), total thyroxine (TT4), total tri-iodothyronine (TT3), adrenocorticotropic hormone (ACTH), cortisol, and growth hormone (GH) in their blood than control children [30]. In Shanghai, China, DLCs detected in maternal breast milk between 2011 and 2012 had a mean of 5.4 pg TEQ/g lipid for PCDD/Fs and a mean of 2.9 pg TEQ/g lipid for dioxin-like PCBs. DLC levels found in breast milk of women living in urban areas were higher than those found in women living in rural areas, and a different distribution was found in each of four geographical regions. Both the distribution and the uptrend were associated with release of these pollutants into the environment resulting from rapid industrialization and urbanization in China. DLC level in breast milk was strongly correlated with maternal age and weakly correlated with consumption of meat and meat products. The estimated daily intake doses for breastfed neonates exceeded the tolerable intake dose cautioned by WHO [31]. A research study in Nanjing, China investigated the association between level of house-dust 39 PCBs collected from urban houses and neurodevelopment in preschool-aged children. The Child Behavior Checklist and the Gesell Development Inventory were used to evaluate child development. This study found that high PCB levels were associated with elevated risk of certain behavior problems and adaptive and gross motor abnormality. This study indicates that exposure to PCBs, especially lower-chlorinated PCBs found in house-dust in urban houses in Nanjing, may increase risk for specific developmental abnormalities [32]. In the Shanghai, China study, concentrations of PCBs and OCPs were analyzed in pooled serum samples from both asthmatic and non-asthmatic children. There were significant differences in 26 PCBs and seven OCPs between case and control groups, with significantly higher levels found in the asthmatic group. Multiple logistic regression models demonstrated that the internal exposure concentrations of a number of POPs (23 PCBs, p,p′-DDE, and α-HCH) were positively

associated with childhood asthma. Some synergistic effects were observed when the children were co-exposed to the chemicals. This study indicates potential relationships between internal exposure concentrations of particular POPs and development of childhood asthma [33]. As described above, monitoring surveys and epidemiological studies in China and India show that serious environmental pollution occurs around electronic-waste processing sites, and indicate that pre-and post-natal exposure to POPs found in such environments may be associated with neurodevelopmental problems and asthma in childhood. Further studies are needed to evaluate effects of exposure to POPs on all aspects of human health, but especially of children, as there is currently very limited information about effects of pollution from electronic-waste processing sites in Asian countries such as India and China (Table 12.5).

12.5 Body Burden of POPs is Related to Exposure Source and Characteristics of the Individual

The use of POPs has been banned or restricted in production and for other purposes in the United States, Europe, Japan, and many additional countries, since the 1970s. Even though many POPs have been prohibited for over 30 years, they are still detected in the environment and in the human body. Recently, epidemiological investigations have been conducted that target the general population, defined as the common population exposed to relatively low levels of POPs. This population is considered to have chronic exposure to low levels of POPs from consumption of certain foods and the use of some common products in their daily lives, without ever having been subject to acute pollution from high levels of POPs, through either accidental poisoning or occupational exposure.

Epidemiological studies conducted in Asian, European, and US populations have found that environmental chemical levels measured in maternal samples are associated with certain demographic, behavioral, dietary, and socioeconomic characteristics. Fish and seafood are the main dietary sources of PCB and PCDD/PCDF exposure in Japan, Taiwan, Nordic countries, and Italy [34], whereas, meat products, dairy products and fish are the main dietary sources in the US, the Netherlands, and Germany [35]. Consequently, it is plausible that exposure sources and their contribution to whole body burden levels of environmental chemicals vary according to specific characteristics of populations in different countries and regions. Detected POPs are positively associated with maternal age and maternal BMI, and inversely associated with history of infant delivery and breast feeding. As a trend, detected POPs increase with advanced age and obesity. Socioeconomic status measures, including education level and household income, are considered indirect indicators of exposure levels of POPs. For example, when high-income pregnant women begin to increase the frequency with which they consume fish and shellfish (the main exposure sources of POPs), they also begin to become exposed to higher levels of POPs.

Table 12.5 Human study evaluated association between environmental pollution and children's health at e-waste site in China

Author	Publication year	Country/ region	Study design	Number of subjects	Exposure	Outcomes	Main findings
Meng	2016	Shanghai, China	Case–control study	124 cases, 109 controls	PCBs and OCPs in pooled sera from asthmatic/ non-asthmatic children,	Asthmatic/non-asthmatic children aged 3–6.	No clear causal relationship between exposure and asthma.Typical POPs might be a risk factor for childhood asthma.
Wang	2015	China	Cross-sectional study	114 houses and children	PCBs in settled house-dust	The child behavior checklist and the Gesell development inventory for children aged 4–16 years.	Post-natal exposure to PCBs was found to have potential adverse effects on the behavior and neurodevelopment of preschool-aged children, although only the univariate test was used.
Xu	2014	Zhejiang Province, China	Cross-sectional study	45 children	PCB, PBDE, and PCDD/F in children serum of 8 years age.	FT3, TT3,FT4, TT4, TSH, ACTH, cortisol, and GH in children serum of 8 years age.	The mean levels of FT3, TT3, TT4, ACTH, cortisol, and GH were higher, whereas the mean levels of FT4 and TSH were lower in the exposed group.

In the National Health and Nutrition Examination Survey (NHANES), a general population study conducted in the U.S., women were found to have detected serum concentrations of PCB 153 that had a median of 15.1 ng/g lipid (in 2003–2004), 11.5 ng/g lipid (in 2005–2006), and 10.3 ng/g lipid (in 2007–2008) [36]. Exposure levels among the general population in East Asia, Japan, were found to be a maternal PCB 153 level of 21.0 ng/g lipid in the Hokkaido study [9], 15.9 ng/g lipid in the Tohoku study [37], and 16.0 ng/g lipid in a study completed in Tokyo [38]. These studies measured levels in pregnant women that seem to be comparable or slightly higher than those observed in the population of U.S. women who participated in the NHANES study. In Korea, detected 153 PCB levels had a median of 9.80 ng/g lw in maternal blood, which was similar to that reported in a study from Peru that was conducted between 2004 and 2005 [39].

Regarding the effect of exposure to PCBs in utero on fetal growth that are observed in human studies, the Hokkaido and Tokyo studies in Japan [9, 38] indicated that no significant associations were found between maternal PCBs level and birth size. Conversely, the Tohoku study found a significant inverse association between concentration of PCBs in cord blood and birth weight in both males and females [40]. Direct comparison of results in the above studies is difficult, due to different sample types and size, and different methods of exposure assessment, statistical method, and selection of confounders. However, it should be noted that the above studies provide conflicting results, despite comparable values of detected PCB levels. One possible explanation is differences in the profiles of study participants, such as maternal smoking status, alcohol consumption, and size of fish and shellfish intake. Fish and shellfish provide essential nutrients, such as n-3 polyunsaturated fatty acids, which have proven beneficial effects on fetal growth. Therefore, consumption of fish and shellfish may neutralize the adverse effects of POPs exposure at the same time they are the primary exposure source [34]. In addition, maternal smoking status is considered an important risk factor for restricted fetal growth, an exposure source of a toxic substance, and it can affect changes in enzyme activity of detoxification metabolism. Importantly, maternal alcohol consumption has adverse effects on fetal growth, disturbs the absorption of nutrition, and also affects changes in detoxification metabolism [9]. Even if comparable exposures to POPs levels are detected, inconsistent results of associations between POPs exposure and outcomes are observed, which are likely due to variation in individual characteristics of participants.

12.6 Exposure Levels and Effects of OCPs

Regarding concentration of OCPs levels in maternal and cord serum, a study in China showed geometric mean levels of p,p′-DDE, HCB, and β-HCH in maternal serum, at 203.54 ng/g, 70.62 ng/g, and 67.67 ng/g, respectively [41]. This study showed that p,p-DDE, total DDT, β-BHC, p,p′-DDT, p,p′-DDD, HCB, and mirex in cord serum were each associated with a decrease in infant birth weight. In a study

conducted in Bangladesh of mean DDT levels in both the breast milk of reproductive age women and the mean levels found in infants exposed to DDT via breast milk, the total estimated daily intake of DDT in the infants did not exceed the tolerable daily intake proposed by WHO (20 ug/kg body wt./day). However, the estimated daily intake of p,p′-DDT exceeded the oral reference dose proposed by the US Environmental Protection Agency [42]. In a study carried out in Sapporo, Japan, the estimated median level of p,p′-DDE in cord serum was 229.1 ng/L. Maternal age was positively associated with the concentration of pesticides (p,p′-DDE, chlordanes group, cis-heptachlorepoxide, β-HCH, and mirex) that was found. Maternal pre-pregnancy body weight was positively associated with concentrations of dieldrin, HCB, β-HCH, Parlar-26, and Parlar-50 [9]. This study found that testosterone was inversely associated with OCP exposure, while DHEA was positively associated with OCP. Estradiol-testosterone and adrenal androgen-glucocorticoid ratios tended to increase with increasing OCP concentrations in the higher quartile, while the testosterone-androstenedione ratio tended to decrease. Sex hormone-binding globulin and prolactin showed an inverse association with OCP exposure. This study suggests that prenatal exposure to OCPs disrupts the reproductive hormones of fetuses in utero in boys, even at relatively low levels [43]. The Sapporo study also reported that prenatal exposure to OCPs, especially cis-heptachlor epoxide, even at low levels, may have an adverse effect on neurodevelopment as tested by the Bayley Scales of Infant Development-II at 18 months of age [44].

In the Children's Health and Environmental Chemicals of Korea (CHECK) cohort, maternal and cord blood serum were taken at delivery, and 19 OCPs and 19 PCBs were measured. The levels of DDTs in both maternal (mean 82.5 ng/g lw) and cord blood serum (mean 77.5 ng/g lw) were comparable to or greater than those reported in Japan approximately 10 years ago. Dairy consumption among the mothers was positively associated with serum levels of several POPs, while tea consumption was negatively associated with serum levels of several POPs [39]. In the CHECK cohort, neurodevelopmental performance was measured with the Bayley Scales of Infant Development-II, the Social Maturity Scale, and the Child Behavior Checklist given to children at 13–24 months of age. Maternal exposure to PCBs was found to be associated with adverse neurodevelopmental performance among children aged 1–2 years [8]. Recent research indicates that pre-and post-natal exposure to OCPs may be associated with sex steroid hormones, birth weight, and neurodevelopment in childhood. More studies of OCP exposure in childhood conducted in Asian countries are needed, as they are currently very limited (Table 12.6).

12.7 Metabolic Syndrome and Obesity Associated with POPs

POPs are bio-accumulated in aquatic and terrestrial organisms, and their pollution levels are increased the higher up the organisms are in the food chain. Predators at the top are often contaminated with the highest levels of POPs as a result of this

Table 12.6 Recent human studies evaluated association between PCB/dioxins/OCPs and children's health among general population in Asia

Author	Publication year	Country/ region	Study design	Number of subjects	Exposure	Outcomes	Main findings
Kim	2018	Korea	Cohort study	46 mother-child pairs	19 PCBs, 19 OCPs, and other chemicals, in urine, whole blood, serum, and/or breast milk of the pregnant or lactating women	BSID-II, social maturity scale, and child behavior checklist at 13–24 months of age	Maternal exposure to several PCBs was associated with adverseneurodevelopmental performances among the children.
Araki	2018	Hokkaido, Japan	Cohort study	202 mother-child pairs	OCPs in maternal blood	LH, FSH, SHBG, prolactin, T, E2, progesterone, cortisol, cortisone, DHEA, androstenedione, inhibin B and INSL3 in cord blood.	Prenatal exposure to OCPs disrupt reproductive hormones of fetuses in utero among boys.
Yamazaki	2018	Hokkaido, Japan	Cohort study	164 mother-child pairs	OCPs in maternal blood	The mental and psychomotor developmental index at 6 and 18 months of age by BSID-II.	Prenatal exposure to OCPs, especially cis-heptachlor epoxide, may have an adverse effect on the neurodevelopment at 18 months of age.
Miyashita	2018	Hokkaido, Japan	Cohort study	183 mother-child pairs	PCDD/PCDFs and PCBs in maternal blood	LH, FSH, SHBG, prolactin, T, E2, progesterone, cortisol, cortisone, DHEA, androstenedione, inhibin B and INSL3 in cord blood.	Prenatal exposure to DLCs alters steroidogenesis (DHEA and T/E2 and adrenal androgen/glucocorticoid ratios) and suppresses the secretion of inhibin B in male
Tatsuta	2017	Tohoku, Japan	Cohort study	489 mother-child pairs	PCBs, methylmercury, and lead	Birth weight	Prenatal PCB exposure reduced birth weight among male and female.
Kishi	2017	Hokkaido, Japan	Cohort study	500 mother-child pairs	OCPs and PCBs in maternal blood	Birth weight, neurodevelopment, allergy, infections, adipocytokine, thyroid hormone	Prenatal exposure to PCDD/PCDFs and OCPs provide negative effects on birth weight, allergy, infections, and neurodevelopment of children.

(continued)

Table 12.6 (continued)

Author	Publication year	Country/ region	Study design	Number of subjects	Exposure	Outcomes	Main findings
Lee	2016	Korea	Cross-sectional study	158 children	OCPs and PCBs in blood of 7–9 aged children.	Diastolic blood pressure. Triglyceride levels, metabolic syndrome score of 7–9 aged children.	Low-dose exposures to PCBs amongchildren in the general population could negatively influence metabolic health, particularly diastolic blood pressure.
Hui	2016	Hong Kong, China	Cohort study	161 mother-child pairs	Dioxins in maternal breast milk	Neurocognitive and intellectual function, including full-scale IQ, fine motor coordination, verbal and non-verbal reasoning, learning ability, and attention at 11 years old	No association between neurocognitive function of children and prenatal dioxin exposure to background levels
Tsuji	2015	Kumamoto, Japan	Case–control study	15 athame, 15 control children	Serum of PCBs in serum of preschool children	The mRNA expression levels of IL-8 and IL-22	IL-8 exhibited significant correlations with the serum levels of individual PCB in asthmatic children
Guo	2014	China	Cross-sectional study	81 newborns	OCPs in the maternal serum and cord blood	Birth weight	Prenatal exposure to DDT, b-BHC, HCB, and mirex were associated with a decrease in birth weight
Hisada	2014	Tokyo, Japan	Cohort study	79 mother-child pairs	16 OH-PCB isomers and of 29 PCB isomers in maternal serum	Birth weight	No associations between OH-PCBs/ PCBs and body size of neonates

food chain biomagnification. Food preferences that include marine mammals create the probability for high exposure to levels of POPs in humans, because marine mammals consumed by humans are the largest predators found on the aquatic food chain. The Inuit people who live in Greenland have traditionally consumed marine mammals, and levels of POPs measured in maternal serum were found to be a median of 105.6 ng/g lipid PCB-153 and 298.9 ng/g p,p -DDE. Previous observations about POPs levels and metabolic syndrome in studies of adults have raised concern about the effects of prenatal exposure to POPs on occurrence of metabolic syndrome and BMI in childhood. The Faroese Birth Cohort study found that prenatal POPs exposures were associated with increased BMI, waist circumference and change in BMI from 5 to 7 years of age, though only in girls with overweight mothers. Moreover, girls born to mothers in the highest quartile of POPs level found in maternal serum during pregnancy were more likely to have high non-fasting insulin levels when compared to girls born to mothers in the lowest quartile. This study suggests that for girls, prenatal exposure to POPs may play a role in later development of metabolic diseases through the impact they have on insulin levels [15].

In one Asian study, the Ewha Birth & Growth Study which is a prospective hospital-based birth cohort in Seoul, Korea, POPs concentration was measured in serum obtained from 214 children, 7–9 years of age. The concentrations of PCBs were significantly associated with increases in diastolic blood pressure and triglyceride levels during a 1-year follow-up. Total PCB levels also showed marginal association with an increase in the continuous metabolic syndrome score compared to baseline. Of the metabolic components, increases in diastolic blood pressure over time showed a notable association with exposure to specific PCBs, but no association was observed with exposure to organochlorine pesticides. This study suggests that low-dose exposures to PCBs in children among the general population can induce adverse effects on metabolic health, particularly in the case of diastolic blood pressure. Increased disease sensitivity during childhood can continue into adulthood, thus, these results support the need for continual assessment of the health impact of POPs [45]. The Ewha Birth & Growth Study investigators also compared the lowest tertile of total PCBs in children aged 7–9 to the other tertiles, and found that participants in the third tertile, had decreased homeostatic model assessment for β-cell function (HOMA-β) values. In a linear mixed model, HOMA-β values were still lower in subjects in the highest compared with the lowest tertile of total PCBs at the 2-year follow-up period. This finding suggests that exposure to POPs might affect insulin secretory function in children, which could lead to an increased risk of developing diabetes. Recent research in Korea indicates that pre-and post-natal exposure to POPs may be associated with insulin secretory function, diastolic blood pressure, and triglyceride levels in childhood. Further studies are needed because there is currently very limited knowledge about insulin secretory function, diastolic blood pressure, and triglyceride levels of children living in Asian countries.

12.8 Conclusions

The use of POPs has been banned or restricted in production and other applications in the United States, Europe, Japan, and many other countries since the 1970s. However, these chemicals are still detected in the environment and in bodies of humans. Recently, environmental pollution in Asian countries has raised concerns about high exposure to POPs in residents and workers, especially in pregnant women and children living in areas surrounding e-waste processing sites. Pre-and early post-natal exposure to high levels of POPs may cause various toxicities including carcinogenicity; teratogenicity; endocrine, immune, and reproductive disruption; and neurobehavioral effects. The general population living in non-industrial areas are chronically exposed to low levels of POPs and epidemiological studies indicate that pre-and early post-natal exposure to even low levels of POPs may be associated with reduced birth weight, changes in sex and reproductive hormones, allergies, infections, neurodevelopmental problems, and metabolic conditions in children. In Asian countries, consumption of fish and shellfish are reported as the primary exposure source of POPs, but it may also protect children from adverse effects of POPs. Even when comparable levels of exposure to POPs are detected in different studies, conflicting results on the associations between POPs and outcomes are observed, due to varying study methods and subject characteristics. Further research is needed because very limited studies exist of pre-and post-natal exposure to POPs and their impacts on children's health in Asian countries.

Acknowledgements This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18gk0110032.

References

1. Van den Berg M, et al. WHO/UNEP global surveys of PCDDs, PCDFs, PCBs and DDTs in human milk and benefit-risk evaluation of breastfeeding. Arch Toxicol. 2017;91(1):83–96.
2. World Health Organization. Endocrine disruptors and child health: possible developmental early effects of endocrine disrupters on child health. Geneva: Public Health and Environment Department, Health Security and Environment Cluster World Health Organization; 2012.
3. Van den Berg M, et al. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci. 2006;93(2):223–41.
4. Gascon M, et al. Effects of persistent organic pollutants on the developing respiratory and immune systems: a systematic review. Environ Int. 2013;52:51–65.
5. Decastro BR, et al. Estrogenic activity of polychlorinated biphenyls present in human tissue and the environment. Environ Sci Technol. 2006;40(8):2819–25.
6. Miyashita C, et al. Sex-related differences in the associations between maternal dioxin-like compounds and reproductive and steroid hormones in cord blood: the Hokkaido study. Environ Int. 2018;117:175–85.
7. Schug TT, et al. Elucidating the links between endocrine disruptors and neurodevelopment. Endocrinology. 2015;156(6):1941–51.

8. Kim S, et al. 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. 2018;624:377–84.
9. Kishi R, et al. The Hokkaido birth cohort study on environment and Children's health: cohort profile-updated 2017. Environ Health Prev Med. 2017;22(1):46.
10. Hui LL, et al. Prenatal dioxin exposure and neurocognitive development in Hong Kong 11-year-old children. Environ Res. 2016;150:205–12.
11. Smialowicz RJ, et al. Relative potency based on hepatic enzyme induction predicts immunosuppressive effects of a mixture of PCDDS/PCDFS and PCBS. Toxicol Appl Pharmacol. 2008;227(3):477–84.
12. Nagayama J, et al. Immunologic effects of perinatal exposure to dioxins, PCBs and organochlorine pesticides in Japanese infants. Chemosphere. 2007;67(9):S393–8.
13. Tsuji M, et al. Association of PCBs and allergies in children. Pestic Biochem Physiol. 2015;120:21–6.
14. Heilmann C, et al. Serum concentrations of antibodies against vaccine toxoids in children exposed perinatally to Immunotoxicants. Environ Health Perspect. 2010;118(10):1434–8.
15. Tang-Peronard JL, et al. Prenatal exposure to persistent organochlorine pollutants is associated with high insulin levels in 5-year-old girls. Environ Res. 2015;142:407–13.
16. Mocarelli P, et al. Paternal concentrations of dioxin and sex ratio of offspring. Lancet. 2000;355(9218):1858–63.
17. Hertz-Picciotto I, et al. A cohort study of in utero polychlorinated biphenyl (PCB) exposures in relation to secondary sex ratio. Environ Health. 2008;7:37.
18. Kido T, et al. Inverse association of highly chlorinated dioxin congeners in maternal breast milk with dehydroepiandrosterone levels in three-year-old Vietnamese children. Sci Total Environ. 2016;550:248–55.
19. Boda H, et al. Prenatal dioxin exposure estimated from dioxins in breast milk and sex hormone levels in umbilical cord blood in Vietnamese newborn infants. Sci Total Environ. 2018;615:1312–8.
20. Pham TT, et al. Perinatal dioxin exposure and the neurodevelopment of Vietnamese toddlers at 1 year of age. Sci Total Environ. 2015;536:575–81.
21. Tai PT, et al. Effects of perinatal dioxin exposure on development of children during the first 3 years of life. J Pediatr. 2016;175:159.
22. Lu YC, Wong PN. PCB poisoning in Japan and Taiwan. Dermatological, medical, and laboratory findings of patients in Taiwan and their treatments. Prog Clin Biol Res. 1984;137:81–115.
23. Aoki Y. Polychlorinated biphenyls, polychloronated dibenzo-p-dioxins, and polychlorinated dibenzofurans as endocrine disrupters - what we have learned from Yusho disease. Environ Res. 2001;86(1):2–11.
24. Tsukimori K, et al. Maternal exposure to high levels of dioxins in relation to birth weight in women affected by Yusho disease. Environ Int. 2012;38(1):79–86.
25. Guo YLL, et al. Yucheng: health effects of prenatal exposure to polychlorinated biphenyls and dibenzofurans. Int Arch Occup Environ Health. 2004;77(3):153–8.
26. Yu ML, et al. The immunologic evaluation of the Yucheng children. Chemosphere. 1998;37(9–12):1855–65.
27. Chao WY, Hsu CC, Guo YL. Middle-ear disease in children exposed prenatally to polychlorinated biphenyls and polychlorinated dibenzofurans. Arch Environ Health. 1997;52(4):257–62.
28. Awasthi AK, Zeng XL, Li JH. Environmental pollution of electronic waste recycling in India: a critical review. Environ Pollut. 2016;211:259–70.
29. Labunska I, et al. Human dietary intake of organohalogen contaminants at e-waste recycling sites in eastern China. Environ Int. 2015;74:209–20.
30. Xu PW, et al. Association of PCB, PBDE and PCDD/F body burdens with hormone levels for children in an e-waste dismantling area of Zhejiang Province, China. Sci Total Environ. 2014;499:55–61.

31. Lu DS, et al. Levels of polychlorinated dibenzo-p-dioxins/furans (PCDD/fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in breast milk in Shanghai, China: a temporal upward trend. Chemosphere. 2015;137:14–24.
32. Wang BL, et al. Levels of polychlorinated biphenyls in settled house dust from urban dwellings in China and their neurodevelopmental effects on preschool-aged children. Sci Total Environ. 2015;505:402–8.
33. Meng G, et al. Internal exposure levels of typical POPs and their associations with childhood asthma in Shanghai, China. Environ Res. 2016;146:125–35.
34. Arisawa K, et al. Dietary patterns and blood levels of PCDDs, PCDFs, and dioxin-like PCBs in 1656 Japanese individuals. Chemosphere. 2011;82(5):656–62.
35. Larsen JC. Risk assessments of polychlorinated dibenzo- p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in food. Mol Nutr Food Res. 2006;50(10):885–96.
36. Sjodin A, et al. Polybrominated diphenyl ethers, polychlorinated biphenyls, and persistent pesticides in serum from the national health and nutrition examination survey: 2003-2008. Environ Sci Technol. 2014;48(1):753–60.
37. Nakamura T, et al. Determination of dioxins and polychlorinated biphenyls in breast milk, maternal blood and cord blood from residents of Tohoku, Japan. Sci Total Environ. 2008;394(1):39–51.
38. Hisada A, et al. Associations between levels of hydroxylated PCBs and PCBs in serum of pregnant women and blood thyroid hormone levels and body size of neonates. Int J Hyg Environ Health. 2014;217(4–5):546–53.
39. Choi S, et al. Current status of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) exposure among mothers and their babies of Korea-CHECK cohort study. Sci Total Environ. 2018;618:674–81.
40. Tatsuta N, et al. Effects of intrauterine exposures to polychlorinated biphenyls, methylmercury, and lead on birth weight in Japanese male and female newborns. Environ Health Prev Med. 2017;22(1):39.
41. Guo H, et al. Prenatal exposure to organochlorine pesticides and infant birth weight in China. Chemosphere. 2014;110:1–7.
42. Haque R, et al. Intake of DDT and its metabolites through food items among reproductive age women in Bangladesh. Chemosphere. 2017;189:744–51.
43. Araki A, et al. Prenatal organochlorine pesticide exposure and the disruption of steroids and reproductive hormones in cord blood: the Hokkaido study. Environ Int. 2018;110:1–13.
44. Yamazaki K, et al. Association between prenatal exposure to organochlorine pesticides and the mental and psychomotor development of infants at ages 6 and 18 months: the Hokkaido study on environment and Children's health. Neurotoxicology. 2018;69:201–8.
45. Lee HA, et al. The effect of exposure to persistent organic pollutants on metabolic health among KOREAN children during a 1-year follow-up. Int J Environ Res Public Health. 2016;13(3):E270.

Chapter 13
Effects of Developmental Exposure to Perfluoroalkyl Substances on Health Outcomes in Pregnant Women and Offspring

Houman Goudarzi and Keiko Yamazaki

Abstract Perfluoroalkyl substances (PFASs) are highly stable and ubiquitous compounds which have been used for more than 60 years in various products; however, their potential health effects have been assessed recently. This chapter summarizes the epidemiological studies regarding the associations of developmental exposure to PFASs with maternal and childhood health outcomes. We have focused on four main health outcomes as follows: fetal and postnatal growth, immunotoxicity, neurodevelopment, and endocrine system outcomes. Although there is a growing literature body in this field, there is still a limited number of prospective cohort studies mainly examining few PFAS types with short follow-up periods. Most studies found an association between PFASs and poor birth outcomes; however, the effects of PFASs on postnatal weight gain and obesity are not well addressed. Several studies reported immunotoxic effects of PFASs in children such as reduced vaccine response, and higher susceptibility to infectious diseases. However, the association with allergic diseases is not clear. Also, effects of PFASs on neurodevelopment are rather inconclusive. On the other hand, PFASs seem to dysregulate the endocrine system such as thyroid and steroid hormones. For the establishment of a causal relationship, further prospective cohort studies with repeated multiple compound exposure assessments are warranted.

H. Goudarzi (✉)
Hokkaido University Center for Environmental and Health Sciences, Sapporo, Hokkaido, Japan

Center for Medical Education and International Relations, Graduate School of Medicine, Hokkaido University, Sapporo, Hokkaido, Japan
e-mail: ghouman@cehs.hokudai.ac.jp

K. Yamazaki
Hokkaido University Center for Environmental and Health Sciences, Sapporo, Hokkaido, Japan

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_13

Keywords Perfluoroalkyl substances · Developmental exposure · Fetal and postnatal growth · Neurodevelopment · Immune system dysfunction · Endocrine system · Emerging contaminants

13.1 Introduction

Perfluoroalkyl substances (PFASs) generally refer to a class of man-made, fully fluorinated compounds with varying carbon chain lengths. Perfluoroalkyls are human-made substances that do not occur naturally in the environment. Their characteristics such as unique surfactant properties have bolstered their use in industrial applications and consumer products for more than 60 years [1, 2]. However, potential health effects of these chemicals by research studies were not accessible to the public until the beginning of this millennium indicating late attention to these compounds [3]. Due to their chemical structure, these compounds are extremely persistent and bioaccumulative [4–6]. Half-lives of PFOS, PFOA, and PFHxS in humans are approximately 5.4, 3.8, and 8.5 years, respectively [7]. Additionally, although PFOS and PFOA are being voluntarily phased out by several industries, they are persistent and still present in older products. Therefore, the long half-lives and resistance to degradation contribute to ubiquitous human exposure. As another concern, the best-known PFAS substitutes with shorter and longer carbon length have been recently used in the industry with no available information on possible health effects.

PFASs have been detected in air, water, and soil in and around fluorochemical facilities [1], and humans are ubiquitously exposed to these chemicals through the oral route (including ingestion of contaminated food and water) and dermal exposure [4, 8, 9]. In contrast to other classical POPs with more lipophilic properties, such as dioxin and polychlorinated biphenyls (PCBs), PFASs do not accumulate in lipids, and they bind to blood proteins [10].

Continued exposure to PFASs and bioaccumulation in human tissues increase human health concerns. Although the developmental effects of PFAS exposure have been examined in several animal studies, the potential effects of PFAS on humans, especially during the perinatal period and childhood, have not been well addressed. PFASs can pass the placenta during pregnancy and reach the fetal circulation [11, 12], and they have been detected both in umbilical cord blood and in human breast milk [1]. Moreover, offspring/maternal blood levels of perfluorinated compounds are greater in humans than in rats or mice [2] which would suggest a significant public health concern. Thus, early life exposure to these compounds may permanently influence developing organ functions and result in noncommunicable diseases throughout the life span.

Therefore, we have reviewed epidemiological studies, mainly birth cohorts, on the potential developmental impact of PFASs on humans from birth to young adulthood including pre- and postnatal growth, neurodevelopment, immunotoxicity, and endocrine endpoints.

13.2 Pre- and Postnatal Growth

We reviewed findings from 28 studies that assessed the potential effects of perinatal PFAS exposures on pre- and postnatal growth, including 20 studies focused on prenatal growth, 5 studies on postnatal growth, and 3 studies on both pre- and postnatal outcomes. Most of them were prospective birth cohort studies; only four studies relied on cross-sectional data (see Table 13.1). The studies mostly examined maternal serum/plasma or cord blood PFASs with a single measurement.

Inoue et al. reported no association between prenatal PFOS exposure and birth weight in a study with a small sample size in Japan [11]. A cross-sectional study reported an inverse association of the cord serum PFOS and PFOA, ponderal index, birth weight, and head circumference [13]. In the Danish National Birth Cohort (DNBC) with large sample size, an inverse association of early-pregnancy PFOA, but not PFOS, with birth weight was observed [14]. Also, in the same population, birth length and abdominal circumference were associated with only PFOA [15]. In addition, the Hokkaido study in Japan found associations of low prenatal PFOS levels, but not for PFOA, with reduced birth weight among female infants [17, 24].

In a community with elevated exposure to electronic waste in China, inverse associations of PFOA with weight and length at birth were reported [21]. In contrast to DNBC, the Aarhus birth cohort from Denmark, with lower exposure levels compared to those in DNBC, did not find any association between PFASs among nulliparous and birth weight or other fetal growth indices [25]. In a Colorado study, infants in the highest tertile of in utero PFHxS, PFOA, and PFNA exposures had lower birth weight and percentage of fat mass.

Furthermore, this study found inverse associations between PFASs and maternal fasting glucose levels during pregnancy. Therefore, the authors suggested that linkage of PFAS exposure and low birth weight and adiposity could be mediated with reduction of maternal glucose supplying fetus during pregnancy [27]. In contrast, some studies did not report associations between PFASs and birth outcomes in Canada [16, 19], Norway [20], and South Korea [26]. Also, researchers in INMA birth cohort did not observe any significant association between PFASs and birth outcomes except a positive association for maternal PFOS and increased risk of small for gestational age (SGA) among male infants [29]. However, these studies are difficult to compare, as they relate to different levels of average exposures and because limited study sample size and narrow ranges of exposure may prevent identification of associations with birth weight. The ATSDR [1] concludes that lowered birth weight is an established adverse effect of PFOS and PFOA.

Table 13.1 Results of epidemiological studies assessing associations of developmental exposure to PFASs with fetal and postnatal growth

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Outcome measures	Primary findings
Fetal growth								
Inoue et al. [11]	Japan	2003	15	Birth cohort (Hokkaido study)	Maternal serum PFOS	The mean of PFOS: 8.8 ng/mL	BW	No association between PFOS and birth weight
Apelberg et al. [13]	Baltimore, USA	2004–2005	293	Cross-sectional	Cord serum PFOS and PFOA	The median of PFOS and PFOA concentration: 5 and 1.6 ng/mL	BW, HC, BL, PI Cord serum lipids: total TG and cholesterol	– Inverse association for PFOS and PFOA with BW, PI, and HC – No associations of PFASs with BL – All associations were independent of cord serum lipid concentrations
Fei et al. [14]	Denmark	1996–2002	1400	Birth cohort (DNBC)	Maternal plasma PFOS and PFOA	The median of PFOS and PFOA concentration: 33.4 and 5.2 ng/mL	BW	Inverse association of PFOA, not PFOS, with BW
Fei et al. [15]	Denmark	1996–2002	1400	Birth cohort (DNBC)	Maternal plasma PFOS and PFOA	The median of PFOS and PFOA concentration: 33.4 and 5.2 ng/mL	PI, BL, HC, AC	– Inverse association for PFOA, but not PFOS, with AC and BL – No significant association of examined PFASs with other birth outcomes
Monroy et al. [16]	Canada	2004–2005	101	Cohort of pregnant women	6 PFASs in maternal and cord serum	The median of PFOS and PFOA concentration: 14.5 and 1.8 ng/mL	BW	No association of examined PFASs with BW

Washino et al. [17]	Japan	2002–2005	428	Birth cohort (Hokkaido study)	Maternal serum PFOS and PFOA	The median of PFOS and PFOA concentration: 5.2 and 1.3 ng/mL	BW, BL, HC, CC	– Inverse association of PFOS, but not PFOA, with BW only among female infants – No association between examined PFASs and other birth outcomes
Stein et al. [18]	Mid-Ohio, Valley, USA	2000–2006	PFOA: 1845 PFOS: 5262	Cross-sectional	Maternal serum PFOS and PFOA	The median of PFOS and PFOA concentration: 12.8 and 21.2 ng/mL	LBW	Weak association between PFOS, but not PFOA, with LBW
Hamm et al. [19]	Canada	2005–2006	252	Birth cohort	Maternal serum PFOS, PFOA, PFHxS	The median PFOS, PFOA, and PFHxS concentration: 7.8, 1.5, 0.97 ng/mL	BW, BW z-score	No association of PFOS, PFOA, and PFHxS with examined outcomes
Whiteworth et al. [20]	Norway	2003–2004	901	Birth cohort (MoBa)	Maternal plasma PFOS and PFOA	The median PFOS and PFOA concentration: 13.0 and 2.2 ng/mL	BW, SGA, LGA	No association between PFASs and birth outcomes
Wu et al. [21]	China	2007	167	Cross-sectional	Maternal serum PFOA	The median of PFOA (in Guiyu): 16.9 ng/mL	BW, BL, PI	Inverse association of PFOA with BW and BL, but not PI
Chen et al. [22]	Taiwan	2004	429	Birth cohort (TBPS)	Cord plasma PFOS, PFOA, PFNA and PFUnDA	The geometric mean for PFOS, PFOA, PFNA, and PFUnDA concentration: 5.9, 1.8, 2.3, and 10.2	BW, BL, HC, PI, SGA	– Inverse association of PFOS with BW and HC – Positive association between PFOS and SGA – No association for other examined PFASs and outcomes

(continued)

Table 13.1 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Outcome measures	Primary findings
Maisonet et al. [23]	Great Britain	1991–1992	447 (girls)	Birth cohort (ALSPAC)	Maternal serum PFOS, PFOA and PFHxS	The median of PFOS, PFOA, and PFHxS concentration: 19.6, 3.7, and 1.6 ng/mL	BW	Inverse association of PFOS, PFOA, and PFHxS with BW
Kishi et al. [24]	Japan	2002–2005	306	Birth cohort (Hokkaido study)	Maternal serum PFOS and PFOA	The median of PFOS and PFOA concentration: 5.6 and 1.4 ng/mL	BW, maternal blood TG and fatty acids	– Inverse association for PFOS, but not PFOA, with BW among female infants – PFOS, but not PFOA, showed inverse association with omega 3 and 6 fatty acids in maternal blood
Bach et al. [25]	Denmark	2008–2013	1507	Birth cohort (Aarhus)	16 PFASs in maternal serum	The median of PFOS and PFOA concentration: 8.3 and 2.0 ng/mL	BW, BL, HC	No association of detected PFASs with birth outcomes
Lee et al. [26]	South Korea	2008	118	Cross-sectional	9 PFASs in cord serum	The median PFOS and PFOA concentration: 0.7 and 1.0 ng/mL	BW	No significant association between examined PFASs and BW

Starling et al. [27]	Colorado, USA	2009–2014	628	Birth cohort (healthy start study)	11 PFASs in maternal serum	The median of PFOS and PFOA concentration: 2.4 and 1.1 ng/mL	– BW, adiposity (body composition) at birth using air displacement plethysmography – Maternal glucose and lipids (TG, total-, HDL-, and non-HDL cholesterol)	– Inverse association of PFOA and PFNA with BW and infant adiposity – Inverse association of PFOA, PFNA, PFDeA, and PFHxS with maternal glucose
Sagiv et al. [28]	Massachusetts, USA	1999–2002	1645	Birth cohort (project viva)	Maternal plasma PFOS, PFOA, PFHxS, and PFNA	The median of PFOS, PFOA, PFHxS, and PFNA concentrations: 25.7, 5.8, 2.4, 0.7 ng/mL	BW-for-gestational-age z score	Weak inverse association of PFOS and PFNA with birth weight-for-gestational-age z-scores
Manzano-Salgado et al. [29]	Spain	2003–2008	1202	Birth cohort (INMA)	Maternal plasma PFOS, PFOA, PFNA, and PFHxS	The median of PFOS, PFOA, PFNA, and PFHxS concentration: 6.0, 2.3, 0.7, and 0.6 ng/mL	BW, BL, HC, LBW, SGA	No association between examined PFASs and birth outcome, except positive association for maternal PFOS and increased risk of SGA in male infants
Li et al. [30]	China	2013	321	Guangzhou birth cohort study	9 PFASs in cord serum	The median of PFOS and PFOA concentration: 3.0 and 1.2 ng/mL	BW, LBW	Inverse association of cord blood PFOS, PFOA, and isomers of PFOS with birth weight especially in male infants

(continued)

Table 13.1 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Outcome measures	Primary findings
Chen et al. [31]	Taiwan	2004–2005	429	Birth cohort (TBPS)	Cord plasma PFOS, PFOA	The median of PFOS and PFOA concentration: 5.7 and 1.9 ng/mL	The age-specific z-scores for weight (WAZ), length/ height (LAZ/HAZ)	Inverse association of PFOS with BW and BL
Buck Louis et al. [32]	USA (12 clinical sites)	2009–2012	2106	Birth cohort	11 PFASs in maternal plasma	The median of PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA concentrations: 0.7, 5.1, 1.9, 0.7, 0.2, 0.1 ng/mL	BW, BL, HC, AC, mid-upper arm, and thigh lengths	– Inverse association of PFOA and BL – Inverse association of PFHxS with umbilical circumference – Inverse association of several PFASs with thigh and arm bone length
Gyllenhammar et al. [33]	Sweden	1996–2011	381	Birth cohort	7 PFASs in maternal serum 3 weeks after delivery	The median of PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA: 2.4, 13, 2.3, 0.4, 0.2, 0.1 ng/g	BW, BL, HC	Inverse associations of maternal levels of PFNA, PFDA, and PFUnDA with birth weight SDS
Postnatal growth								
Andersen et al. [34]	Denmark	1996–2002	1010	Birth cohort (DNBC)	Maternal plasma PFOS and PFOA	The median of PFOS and PFOA concentration: 33.4 and 5.2 ng/mL	Weight, length and BMI at 5 and 12 months of age	Inverse association of maternal PFOS and PFOA with weight and BMI at 5 and 12 months of age

Halldorsoon et al. [35]	Denmark	1988–1989	665	Birth cohort (Aarhus)	Maternal serum PFOS, PFOA, PFOSA, and PFNA	The median of PFOA, PFOS, PFOSA, and PFNA concentration: 3.7, 21.5, 1.1, and 0.3 ng/mL	BMI, waist circumference, insulin, and adipokines in offspring at age 20	– Positive association of PFOA with overweight/obesity and waist circumference of female offspring – Association of PFOA with increased serum insulin and leptin but decreased adiponectin levels – No association for PFOS, PFNA, and PFOSA with examined outcomes
Maisonet et al. [23]	Great Britain	1991–1992	447 (girls)	Birth cohort (ALSPAC)	Maternal serum PFOS, PFOA, and PFHxS	The median of PFOS, PFOA and PFHxS concentration: 19.6, 3.7, and 1.6 ng/mL	Weight at weight at 2, 9 and 20 months of age	Positive association of PFASs with weight at 20 months of age among girls
Andersen et al. [36]	Denmark	1996–2002	811	Birth cohort (DNBC)	Maternal plasma PFOS and PFOA	The median of PFOS and PFOA concentration: 33.8 and 5.2 ng/mL	Children's body mass index, waist circumference, and risk of overweight at 7 years of age	No association of PFASs with anthropometry at 7 years of age
Braun et al. [37]	Ohio, USA	2003–2006	204	Birth cohort (HOME study)	Maternal serum PFOS, PFOA, PFNA, and PFHxS	The median of PFOS, PFOA, PFNA, and PFHxS concentration: 13.0, 5.3, 0.9, 1.4 ng/mL	BMI and waist circumference at age 8, and BMI between 2 and 8 years of age	Association of PFOA with greater adiposity at 8 years and a more rapid increase in BMI between 2 and 8 years

(continued)

Table 13.1 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Outcome measures	Primary findings
Mora et al. [38]	Massachusetts, USA	1999–2002	1006 (early childhood), 876 (mid-childhood)	Birth cohort (Project Viva)	Maternal plasma PFOS, PFOA, PFNA, and PFHxS	The median of PFOS, PFOA, PFNA, and PFHxS concentration: 24.8, 5.6, 0.6, and 2.4 ng/mL	Anthropometric and dual X-ray absorptiometry measurements in early (median, 3.2 years) and mid-childhood (median 7.7 years)	Association of maternal PFASs with small increase in BMI, subscapular and triceps skinfold thickness, and total fat mass index in mid-childhood among girls
Chen et al. [31]	Taiwan	2004–2005	429	Birth cohort (TBPS)	Cord plasma PFOS, PFOA	The median for PFOS and PFOA concentration: 5.7 and 1.9 ng/mL	The age-specific z-scores for weight, length/height, and BMI until 108 months of age	– Inverse association of PFOS with age-specific z-scores for weight and BMI during time span of 6–12 and 12–24 months (girls) – Association of PFOS with the age-specific z-score for BMI during a period of 60–108 months of age (girls)
Gyllenhammar et al. [33]	Sweden	1996–2011	381	Birth cohort	7 PFASs in maternal serum 3 weeks after delivery	The median of PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA: 2.4, 13, 2.3, 0.4, 0.2, 0.1 ng/g	Weight, length/height, and head circumference at 3, 6, 12, and 18 months and 3, 4, and 5 years of age	– No association of PFASs with weight and length SDS – Association of BMI SDS with PFOA, PFNA, and PFHxS at 3 and 4 years of age, and with PFOS at 4 and 5 years of age

BW birth weight, *BL* birth length, *PI* ponderal index, *HC* head circumference, *AC* abdominal circumference, *CC* chest circumference, *LBW* low birth weight, *SGA* small for gestational age, *LGA* large for gestational age, *TG* triglyceride, *SDS* standard deviation scores

ALSPAC Avon Longitudinal Study of Parents and Children, *DNBC* Danish National Birth Cohort, *MoBa* Norwegian Mother and Child Cohort Study, *TBPS*: Taiwan Birth Panel Study

Few studies have reported associations between PFASs and low birth weight (LBW). One study among Mid-Ohio Valley residents in the USA (the C8 Health project) reported a weak but significant positive association between maternal PFOS concentrations and LBW risk [18]; however, several other studies did not find any associations in Denmark [14], Spain [29], and China [30]. It is not clear if the absence of significant associations is mainly due to limited statistical power to identify a small effect.

There are eight studies focusing on the effect of PFASs on postnatal growth. Of those, three studies examined pre- and postnatal growth in the same population [23, 31, 33]. A British study among girls reported an inverse dose-response between in utero PFOS, PFOA, and PFHxS exposures and birth weight; however, at 20 months of age, girls born to mothers in the upper tertile of PFOS concentrations were heavier than those in the lower tertile [23]. The Taiwan Birth Panel Study (TBPS) reported the association of PFOS in cord plasma with reduced birth weight and head circumference and positive association with SGA [22], but in a longer follow-up they found the positive association of cord plasma PFOS with BMI among girls at 60–108 months of age [31]. However, DNBC researchers reported that in utero PFOS and PFOA exposure was associated with the offspring weight at ages 5 and 12 months [34], although there was no association with anthropometric measures at age 7 [36]. In Sweden, maternal levels of PFNA, PFDA, and PFUnDA, measured in maternal serum samples collected 3 weeks after delivery, were associated with reduced birth weight standard deviation scores. The study followed up children at 3, 6, 12, 18, 36, 48, and 60 months of age and found that BMI SDS was significantly associated with PFOA, PFNA, and PFHxS at 3 and 4 years of age, and with PFOS at 4 and 5 years of age [33]. Researchers in the HOME birth cohort study reported associations between prenatal PFOA, but not PFOS, PFNA, and PFHxS, with higher BMI gains from 2 to 8 years and greater adiposity at age 8 years among children in Cincinnati [37]. The Project Viva birth cohort reported association of prenatal PFAS levels with higher BMI and a total fat mass index (using X-ray absorptiometry) in mid-childhood among girls [38]. Also, they found associations of early-pregnancy PFOS and PFNA levels with reduced birth weight-for-gestational age z scores [28]. A study in Denmark assessed long-term effects of in utero PFAS exposure risk of overweight at 20 years of age and reported that only maternal PFOA, but not PFOS and PFNA, was associated with postnatal risk of overweight or obesity, and higher waist circumference only among girls [35]. The study also found that in utero PFOA exposure was positively correlated with serum insulin and negatively with leptin in female offspring [35].

Taken together, despite some inconsistency, a preponderance of studies suggested that PFAS affects pre- and postnatal growth. Further studies with larger sample sizes and longer follow-up for longitudinal observations are still needed to clarify the impact of PFASs on postnatal life growth and metabolic outcomes.

13.3 Neurodevelopment

We reviewed 26 epidemiological studies that assessed the potential effects of PFASs on neurodevelopment, mostly prospective cohort studies following up children from birth up to young adulthood (Table 13.2). We categorized outcome as follows: (a) developmental milestones in infancy (5 studies), (b) cognitive functions (8 studies), and (c) neurodevelopmental disorders (ADHD, ASD, etc.) and behavior problems (13 studies). Most studies examined prenatal PFAS concentrations and were conducted in Northern Europe, the USA, and East Asia.

13.3.1 Neurodevelopment in Infants

Five prospective birth cohort studies examined associations between perinatal PFAS exposures and developmental milestones in infants. Three of five studies that evaluated neurodevelopmental indicators in early infancy (below 2 years of age) reported an inverse association between prenatal PFAS levels and development. A multicenter study in Cincinnati examined the association of relatively high PFAS exposure levels with infant neurobehavior at approximately 5 weeks of age using the Neonatal Intensive Care Unit Network Scale (NNNS). PFOA levels were associated with hypotonicity in infants [39]. In the Hokkaido study with low PFAS exposure levels, neurodevelopment indices were examined by face-to-face interview and examination by professional staffs using the Bayley Scales (BSID II) at ages 6 and 18 months of age. The researcher found that female infants born to mothers with in utero concentrations of PFOA, but not PFOS, in the fourth quartile had significantly lower mental development index scores than female infants born to mothers with concentrations of PFOA in the first quartile. However, no association was found between PFASs and neurodevelopment scales in boys at the same age. Also, no association of prenatal PFASs with mental or psychomotor development scales was found at age 18 months for both sexes [40]. The TBPS reported a dose-response relationship of cord blood PFOS, but not PFOA, with low developmental quotients of gross-motor subdomain at 2 years of age; however, there is no convincing association of PFASs with cognitive, language, social, and self-help domains [41]. In contrast, two studies in Norway and Denmark reported no association of PFAS exposure and neurodevelopment in infants [42, 64]. The DNBC reported no convincing associations of early-pregnancy PFOS and PFOA concentrations with motor or mental development milestones reported by the mothers at ages 6 and 18 months except weak association of PFOS with a later age of being able to sit alone [64]. Finally, a birth cohort in Norway also found no associations of PFOS and PFOA levels measured in breast milk and neuropsychological development at 12 and 24 months [42].

Table 13.2 Summary of studies assessing association between developmental exposure to PFASs and neurodevelopmental outcomes

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Developmental milestones in infancy									
Donauer et al. [39]	Cincinnati, USA	2003–2006	349	Cohort	Prenatal PFOS and PFOA in maternal serum	The geometric mean of PFOS and PFOA were 13.25 and 5.49 ng/mL	5 weeks	Neonatal intensive care unit network neurobehavioral scale (NNNS) administered	– No association of PFOS and PFOA with the 11 NNNS outcomes – A tenfold increase in prenatal PFOA increased the odds of being hypotonic in latent profile analysis
Fei et al. [15]	Denmark	1996–2002	1400	Cohort	Prenatal PFOS and PFOA in maternal plasma	The mean of PFOS and PFOA were 35.3 and 5.6 ng/mL	6 and 18 months	Developmental milestones within the mental and motor domains reported by mothers	No convincing association of PFOA or PFOS with motor or mental development in early infancy
Goudarzi et al. [40]	Japan	2002–2005	173	Cohort	Prenatal PFOS and PFOA in maternal plasma	The median of PFOS and PFOA were 5.7 and 1.2 ng/mL	6 and 18 months	The Bayley Scales of Infant Development (BSID II)	– Prenatal PFOA concentrations were associated with the MDI of female (but not male) infants only at 6 months of age – No associations of PFOS with any examined outcome
Chen et al. [41]	Taiwan	2004–2005	239	Cohort	PFOS and PFOA in cord blood	The mean of PFOS and PFOA were 7.0 and 2.5 ng/mL	2 years	Developmental inventory for infants and toddlers completed by physical therapists	Association of PFOS with adverse performance on the whole test and the domains related to development, especially in the gross-motor subdomain

(continued)

Table 13.2 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Forns et al. [42]	Norway	2003–2009	843	Cohort	PFOS and PFOA in breast milk	The median of PFOS and PFOA were 110 and 40 ng/L	6 and 24 months	Ages and Stages Questionnaire (ASQ-II)	No association between perinatal PFOS and PFOA exposure and early neuropsychological development
Cognitive functions									
Gump et al. [43]	Oswego County, NY	–	83	Cross-sectional	11 PFASs in children blood	PFOS: 8.8, PFOA: 3.3, PFNA: 0.7, PFDA: 0.26, PFHxS: 3.7, PFOSA: 0.6 ng/mL median	9–11 years	Differential reinforcement of low rates of responding (DRL) task to assess response inhibition	Associations between multiple PFASs with behavioral inhibition deficits in children
Stein et al. [44]	Mid-Ohio Valley, USA	2005–2006	320	Cohort	Estimated in utero PFOA exposure, measured childhood PFOA serum age 2–8 years	The mean (ng/mL) of prenatal PFOA 115.9, and childhood PFOA 91.9	6–12 years	A battery of tests including intelligence quotient (IQ), reading and math skills, language, memory and learning, visual-spatial processing, and attention measured by certified examiners	Children in the highest as compared with lowest quartile of estimated in utero PFOA had increases in full scale IQ and decreases in characteristics of ADHD
Wang et al. [45]	Taiwan	2000–2001	120	Cohort	Seven prenatal PFASs in maternal serum	The median (ng/mL) of PFOS 13.25, PFOA 2.50, PFUnDA 3.42, PFNA 1.59, PFHxS 0.69, PFDeA 0.44, PFDoDA 0.38	Age 5 and 8	Wechsler preschool and primary Scale of intelligence-revised (WPPSI-R) administered by trained psychologists	Association of PFUnDA and PFNA (long-chain PFASs) with decreased childhood IQ test scores

Vuong et al. [46]	Cincinnati, USA	2003–2006	256	Cohort	Prenatal PFOA, PFOS, PFHxS, PFNA, and PFDeA	The median (ng/mL) of PFOS 13.2, PFOA 5.4, PFHxS 1.5, PFNA 0.9, PFDeA 0.2	Ages 5 and 8	Executive function assessed with the parent-rated behavior rating inventory of executive function (BRIEF)	Association of PFOS with poorer behavior regulation, meta-cognition, and global executive functioning
Harris et al. [47]	Eastern Massachusetts, USA	1999–2002	631–971	Cohort and cross-sectional	8 PFASs from prenatal and mid-childhood (median age 7.7 years) plasma	The median (ng/mL) of prenatal: PFOA 5.6, PFOS 24.9, PFHxS 2.4, PFNA 0.6, EtFOSAA 1.2, MeFOSAA 1.9 Childhood: PFOA 4.4, PFOS 6.2, PFHxS 1.9, PFNA 1.5, MeFOSAA 0.3, PFDeA0.3	Early childhood (median age3.2 years) and mid-childhood (median age 7.7 years)	In early childhood, pea body picture vocabulary test, third edition (PPVT-III) to assess vocabulary comprehension, and the wide range assessment of visual motor abilities (WRAVMA) to assess visual-motor, fine motor, and visuospatial skills In mid-childhood, the Kaufman brief intelligence test, second edition (KBIT-2) to assess verbal and nonverbal intelligence, WRAVMA drawing subtest to assess visual-motor skills, visual memory index of the wide range assessment of memory and learning, second edition (WRAML2) to assess visual memory	Association of prenatal PFAS with both better and worse cognitive performance

(continued)

Table 13.2 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Liew et al. [48]	Denmark	1996–2002	1592	Cohort	16 prenatal PFASs in maternal plasma	The median (ng/mL) of PFOS 28.10, PFOA 4.28, PFHxS 1.07, PFNA 0.46, PFHpS 0.37, PFDA 0.17, PFOSA 2.32, PFHpA 0.07	Age 5	Wechsler preschool and primary Scale of intelligence-revised (WPPSI-R)	No convincing association between PFASs and child IQ scores at 5 years of age
Vuong et al. [49]	Cincinnati, USA Cincinnati, USA	2003–2006 2003–2006	218 208	Cohort and cross-sectional Cohort and cross-sectional	4 PFASs from prenatal and 3- and 8-year child serum	The median (ng/mL) of prenatal: PFOA 5.2, PFOS 12.9, PFHxS 1.3, PFNA 0.9, 3y: PFOA 5.4, PFOS 6.2, PFHxS 1.9, PFNA 1.3, 8y: PFOA 2.5, PFOS 3.6, PFHxS 0.9, PFNA 0.5	Age 8	Conners' continuous performance test–II (CPT-II) to assess attentional functioning and impulsivity, virtual Morris water maze (VMWM) to assess partial learning and memory retrieval performance	– Mixed findings for prenatal and childhood PFAS concentrations and visual spatial abilities – No enough evidence to support association of PFAS with visual spatial abilities as assessed by the VMWM or CPT-II measures of inattention or impulsivity in children at age 8 years
					6 PFASs from 3 and 8y child serum	The median (ng/mL) of 3 years: PFOA 5.4, PFOS 6.1, PFHxS 1.2, PFNA 1.9, PFDA 0.2, me-PFOSA-AcOH 0.3, 8 years: PFOA 2.4, PFOS 3.6, PFHxS 0.7, PFNA 1.2, PFDA 0.2, me-PFOSA-AcOH 0.2	Age 8	Executive function assessed with the parent-rated behavior rating inventory of executive function (BRIEF)	PFNA and PFOA at 8 years, but not 3 years, associated to poorer executive function at 8 years

Zhang et al. [50]	Cincinnati, USA	2003–2006	167	Cohort and cross-sectional	4 PFASs from prenatal and 3- and 8-year child serum	The median (ng/mL) of prenatal: PFOA 5.4, PFOS 13.0, PFHxS 1.5, PFNA 0.9, 3 years: PFOA 5.5, PFOS 6.6, PFHxS 1.9, PFNA 1.2, 8 years: PFOA 2.4, PFOS 3.6, PFHxS 1.2, PFNA 0.7	Age 5 and 8	Woodcock-Johnson tests of achievement III (WJ-III) to assess children's reading skills at age 5 and wide range achievement test 4 (WRAT-4) at age 8 years	– Positive association of prenatal and childhood serum PFOA and PFOS and with better children's reading skills at ages 5 and 8 years – No association between serum PFHxS and reading skills
ADHD, ASD, and behaviors in childhood									
Hoffman et al. [51]	USA	1999–2000 2003–2004	Total: 571 ADHD case: 48	Cross-sectional	PFOS, PFOA, PFNA, PFHxS in childhood serum ≥12 years of age	The median (μg/L) of PFOS 22.6, PFOA 4.4, PFHxS 2.2, PFNA 0.6	12–15 years	Parental report of previous ADHD diagnosis and medication	Association of higher serum PFAS levels with higher odds ratio of ADHD in children
Fei and Olsen [52]	Denmark	1996–2002	787 for SDQ; 526 for DCDQ	Cohort	Prenatal PFOS and PFOA in maternal plasma	The median of PFOS and PFOA were 34.4 and 5.4 ng/mL	7 years	The strengths and difficulties questionnaire (SDQ) and the developmental coordination disorder questionnaire (DCDQ) reported by mothers	No association between prenatal levels of PFOS or PFOA with SDQ and DCDQ scores
Stein and Savitz [53]	Mid-Ohio Valley, USA	2005–2006	12,016	Cross-sectional	PFOA, PFOS, PFHxS, PFNA in childhood serum	PFOA: 66.3, PFOS:22.9, PFHxS: 9.3, PFNA: 1.7 ng/mL mean	5–18 years	Diagnosis of ADHD as reported in the questionnaire	– Reduced odds of ADHD at the highest PFOA exposure level – Positive associations of other PFASs with ADHD, particularly PFHxS

(continued)

Table 13.2 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Braun et al. [54]	Cincinnati, USA	2003–2006	175	Cohort	Prenatal PFOS, PFOA, PFNA and PFHxS in maternal serum	The median (ng/mL) of PFOS 13, PFOA 5.5, PFNA 0.9, PFHxS 1.6	4–5 years	Mothers completed the Social Responsiveness Scale (SRS), a measure of autistic behaviors	Association of PFOA with less autistic behaviors
Ode et al. [55]	Sweden	1978–2000	206 ADHD cases and 206 controls	Cohort	PFOS, PFOA, or PFNA in umbilical cord serum	The median of PFOS and PFOA in controls were 6.77 and 1.83 ng/mL	Most children Diagnosed ages 8–12 years	ADHD were diagnosed by experienced clinicians Using the diagnostic and statistical manual of mental disorders (DSM)	No associations of prenatal PFOS, PFOA, and PFNA with childhood ADHD
Stein et al. [56]	Mid-Ohio Valley, USA	2005–2006	321	Cohort	Childhood PFOA serum concentration at age 2–8 years	The median of PFOA was 35.1 ng/mL	6–12 years	Mother and teacher reports of executive function (BRIEF), ADHD-like behavior (Conners' scales), and behavioral problems (behavior assessment system for children)	– Overall, neither reports from mothers nor teachers provided clear associations between PFOA exposure and child behavior. – Mother reports, however, did suggest favorable associations between exposure and behavior among boys and adverse associations among girls

Strom et al. [57]	Denmark	1988–1989	876	Cohort	Prenatal PFOS and PFOA in maternal serum	The median (ng/mL) of PFOS 21.4, PFOA 3.7	Up to age 20	Diagnosis and medication for ADHD or depression. Scholastic achievement defined as mean grade on a standardized written examination given in the ninth grade	No association of prenatal exposure to PFOS and PFOA with offspring behavioral and affective disorders or scholastic achievement
Hoyer et al. [58]	Greenland, Kharkiv (Ukraine) and Warsaw (Poland)	2002–2004	1106	Cohort	Prenatal PFOS and PFOA in maternal plasma	The median (ng/mL) of PFOS 20.3 in Greenland, 5.0 in Ukraine, and 8.0 in Poland; PFOA 1.8 in Greenland, 1.0 in Ukraine, and 2.7 in Poland	5–9 years	DCDQ and SDQ	A small-to-moderate effect of prenatal exposure to PFOS and PFOA on children's neurobehavioral development, specifically in hyperactive behavior
Liew et al. [59]	Denmark	1996–2002	890 ADHD, 301 autism, and 550 controls	Cohort	Six prenatal PFASs in maternal plasma	The median (ng/mL) of PFOS 27.40, PFOA 4.00, PFHxS 0.92, PFNA 0.43, PFHpS 0.30, and PFDA 0.17 in the controls	Average 10.7-year follow-up	Medical records from national registers (ICD-10 F90.0 for ADHD and F84.0 for childhood autism)	No convincing evidence on a link between prenatal exposure to PFASs and the risk of ADHD or autism in children
Lien et al. [60]	Taiwan	2004–2005	282 with complete information	Cohort	PFOA, PFOS, PFNA and PFUA in umbilical cord blood	The mean of PFOA, PFOS, PFNA, and PFUA were 1.55, 4.79, 4.49, and 7.96 ng/mL	7 years	Parents completed the Swanson, Nolan, and Pelham IV scale (SNAP-IV), the child behavior checklist (CBCL), and the SDQ	– Inverse association of PFNA with inattention and oppositional defiant disorder of SNAP-IV, and hyperactivity/inattention of SDQ. – No association of PFOA, PFOS, or PFUA with ADHD symptoms

(continued)

Table 13.2 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Oulhote et al. [61]	Faroe Islands	1997–2000	656	Cohort	Five PFASs in prenatal maternal serum, and serum from children aged 5–7 years	The median (ng/mL) of prenatal PFOS 27.35, PFOA 3.34, PFHxS 8.43, PFNA 0.61, PFDA 0.29.	5 and 7 years	Parent-reported SDQ	Association of higher childhood serum PFOA, PFNA, and PFDA concentrations at ages 5 and 7 years, but not prenatally, with behavioral problems at age 7
Quaak et al. [62]	Netherlands	2011–2013	76	Cohort	PFOA, PFOS, and 5 PFASs in cord plasma	The median (ng/mL) of PFOA 0.87, PFOS 1.6, PFHxS 0.145, PFHpS 0.031, PFNA 0.14, PFDA 0.046, PFUnDA 0.(0275), sum PFASs 2.907	18 months	Child behavior checklist 1.5–5 (CBCL) to assess the behavioral development of the child	– Inverse association of PFOA with externalizing behavior in boys – Association of higher levels of ΣPFAS exposure with lower scores on the externalizing problem scale in girls
Lyall et al. [63]	Southern California, USA	2000–2003	ASD:553 Intellectual disability: 189 Control: 433	Cohort and nested case-control	Prenatal 8 PFASs in maternal serum	The GM (ng/mL) of: control group: Et-PFOSA-AcOH 0.7, Me-PFOSA-AcOH 1.1, PFDeA 0.1, PFHxS 1.3, PFNA 0.6, PFOA 3.6, PFOS 17.9, PFOSA 0.1, ASD group: Et-PFOSA-AcOH 0.6, me-PFOSA-AcOH 1.1, PFDeA 0.1, PFHxS 1.4, PFNA 0.6, PFOA 3.5, PFOS 17.5, PFOSA 0.1	4.5–9 years	– Trained medical record abstractors reviewed and compiled diagnostic and clinical data for children receiving services for ASD or intellectual disability – Expert clinical review of abstracted data was then conducted by a developmental pediatrician to confirm the ASD diagnoses using diagnostic and statistical manual of mental disorders, fourth edition (DSM-IV-TR) criteria.	Prenatal PFAS exposure levels were lower in ASD and intellectual disability groups relative to general controls

13.3.2 Cognitive Function

Eight studies examined neuropsychological functions as outcomes in children elder than 2 years reported inconsistent results. A study found the inverse association of prenatal perfluoroundecanoic acid (PFUnDA) with performance IQ scores in Taiwanese children at age 5. In the same population, the researchers reported the inverse association of verbal IQ scores with prenatal PFNA at age 8 [45]. A small cross-sectional study in N.Y. found the association of PFHxS, PFOSA, PFOS, PFNA, and PFDA with behavioral inhibition deficits in children between 9 and 11 years [43]. A cohort found that prenatal PFOS, but not PFOA, was associated with poorer executive function at ages 5 and 8 in Cincinnati [46]. Additionally, the same study measured children's exposure levels at ages 3 and 8 and reported that only PFNA and PFOA at 8 years are associated with executive function deficits at 8 years [49]. In contrast, the same cohort found that prenatal and postnatal serum PFOA, PFOS, and PFNA concentrations were positively associated with reading abilities at ages 5 and 8 years [50]. A Danish study in Denmark also found no consistent evidence to suggest prenatal exposure to PFASs to be associated with child IQ scores at 5 years of age [48]. Consistently, the C8 Health Study found that exposure modeling-generated individual estimates of maternal serum PFOA levels were positively associated with full-scale IQ in children of 6–12 years [44]. Another study in Cincinnati did not support associations between pre- and postnatal PFAS with visual-spatial abilities [49]. Lastly, a study in Eastern Massachusetts, USA, reported that prenatal PFASs were associated with both better and worse cognitive scores. Also, childhood PFASs were associated cross-sectionally with lower visual-motor abilities [47].

13.3.3 Neurodevelopmental Disorder and Behavioral Problem

Thirteen studies focused on the developmental disorder (e.g., ADHD, ASD, etc.) and behavioral problems in childhood associated with PFAS exposures. Most studies found no convincing evidence to suggest that prenatal PFAS exposure increases the risk of ADHD or ASD [55, 57, 59].

A small Dutch cohort with small sample size examining infants at 18 months found the prenatal negative association of PFOA and externalizing behavior in boys; in contrast, ΣPFAS exposure in girls was associated with lower scores on the Externalizing Problem Scale [62]. A Taiwanese cohort reported that cord blood PFNA, but not PFOS, PFOA, and PFUnDA, was associated with ADHD-related neurobehavioral symptoms among 7-year-old children [60]. INUENDO birth cohort study, with pooled analysis of data in Ukraine (Greenland and Kharkiv) and Poland (Warsaw) examining neurodevelopment of children between 5 and 9 years old, reported a small-to-moderate association of prenatal PFOA and hyperactive behavior in total samples mainly in Greenland, along with positive association of PFOS

and behavioral problems [58]. Among Faroese children, postnatal PFOA, PFNA, and PFDA, but not prenatally, were positively associated with patient-reported behavioral problems at age 7 [61]. The DNBC also found no convincing association between early-pregnancy PFOS or PFOA and behavioral and motor coordination problems at age 7 [52].

The C8 health study measured serum PFOA levels in a highly exposed community among children aged 2–8 years highly exposed to PFOA and reported the association of PFOA and improved executive function and ADHD-like problems among boys but also found adverse outcomes among girls assessed in the follow-up about 3–4 years after exposures [56]. Another data from the C8 Health Project among non-Hispanic white children at ages 5–18 years with cross-sectional design showed association of very high childhood PFOA exposure levels with reduced risk of ADHD, and in contrast positive association of PFASs, particularly PFHxS, and ADHD [53]. Another cross-sectional study from NHANES found an increased risk of parental-reported ADHD with PFOA, PFOS, and PFHxS among children at 12–15 years of age [51]. There are a few studies regarding the effects of PFASs on ASD diagnosis and ASD-related symptoms. A study from DNBC using national registry examined the association of prenatal PFASs with childhood ADHD and autism with a nested case-control design and reported no convincing evidence for the link of PFAS with ADHD and autism [59]. Additionally, the Home study in Cincinnati observed no convincing association between prenatal exposure to PFASs and autistic behavior among 4- and 5-year-old children in Cincinnati [54]. Early Markers for Autism (EMA) study in southern California with nested case-control study design among 4.5- to 9-year-old children reported that ASD was not significantly associated with prenatal concentrations of eight examined PFASs, except borderline significant inverse associations of PFOA and PFOS with ASD [63]. A Danish study reported no association of prenatal exposure to PFASs and offspring ADHD, depression, and scholastic achievement over a follow-up period of up to 22 years [57]. Lastly, a study from DNBC found a dose-response increase in risks for cerebral palsy with in utero exposure to PFASs with a case-cohort design in Denmark [65].

As a summary, a growing number of recent studies examined the neurodevelopmental effects of PFASs among children. However, the results across studies are inconsistent, and it is not clear how robust the studies are in regard to confounding. Most of the studies reported null associations, while some studies found positive or inverse associations. The comparison of different studies is difficult because of applying different timing of exposure assessment, various battery tests to assess different neurodevelopment domains, and a wide range of ages for neurodevelopmental examinations. Most studies have not examined multiple exposure assessments of PFASs and other POPs. Therefore, more prospective studies with larger sample sizes using different battery tests examining various neurodevelopment domains and longer follow-up periods are warranted, preferably with better documentation of relevant cofactors.

13.4 Immunotoxicity

Excluding studies conducted in occupational settings and in adults, animal study, and review articles, we have reviewed 30 epidemiological studies regarding the effects of PFASs on immune system endpoints among children and adolescents in this section. Most studies had prospective birth cohort design, five studies were cross-sectional studies, and five were case-control studies. We categorize the outcomes as follows: (1) vaccine response, (2) infectious diseases, (3) allergic conditions and IgE, and (4) other immune function biomarkers.

13.4.1 Vaccine Response and Infectious Diseases

Six epidemiological studies examined the effects of PFASs on vaccine response, three studies focused on infectious diseases, and two studies looked at vaccine response and infectious diseases at the same time (Table 13.3). All studies except one cross-sectional study were prospective birth cohort studies.

A Norwegian study found an inverse association between in utero exposure to PFASs and anti-rubella antibody levels in the children's serum at age 3. However, associations of prenatal PFAS exposure with influenza, measles, and tetanus vaccine antibody levels at age 3 were not conclusive in this study [69]. Another study from the same cohort identified a toxicogenomics profile of 52 PFAS exposure-associated genes, including several immunomodulatory genes, that were in common with rubella antibody titer-related genes and/or common cold episodes [72].

In a Faroese birth cohort, doubling of the estimated exposures to perfluorinated compounds in infancy was associated with a 19–29% decrease in children's tetanus antibody concentrations at age 5 [75]. In the same cohort, another study examined prenatal and postnatal PFAS exposure and showed negative associations of PFAS concentrations at age 5 with tetanus and diphtheria antibody concentrations at ages 5 and 7 [67]. Prenatal PFAS concentrations had less clear associations with childhood serum vaccine antibody concentrations [67]. With longer follow-up of children and measurement of PFAS and vaccine antibody concentrations at ages 7 and 13, Grandjean et al. reported that diphtheria antibody concentrations, but not tetanus antibody concentrations, decreased at high PFAS concentrations at 13 and 7 years [74]. A study in NHANES with cross-sectional design assessed association of serum PFASs with measles, mumps, and rubella antibody concentrations at ages 12–19 and found the association of PFASs with reduced mumps and rubella antibody concentrations [86]. In contrast, another NHANES study found no association between PFASs and rubella immunity at ages 12–18 [76]; however, they observed inverse associations between PFASs and rubella antibody titers in adults at 19–49 years. Of note, these cross-sectional studies did not take into regard important predictors, such as time since vaccination.

Table 13.3 Summary of studies assessing associations of developmental exposure to PFASs with immune outcomes

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Vaccine response and infectious diseases									
Fei et al. [66]	Denmark	1996–2002	1400	Cohort	Maternal PFOA and PFOS	The mean concentration was 5.6 ng/mL for PFOA and 35.3 ng/mL for PFOS	0–10 years	– Hospitalizations for infection	– No association between prenatal exposure to PFOA and PFOS with hospitalizations due to infections – Higher risk of hospitalizations in girls and lower risk in boys associated with PFASs exposure
Grandjean et al. [67]	Faroe Islands	1997–2000	587	Cohort	Maternal and 5-year-old PFOA, PFOS, PFHxS, PFDA, and PFNA	Geometric mean concentrations respectively for PFOA, PFOS, PFHxS, PFDA, and PFNA were: maternal: 3.2, 27.3, 4.4, 0.3, and 0.6 ng/mL; 5-years: 4.1, 16.7, 0.6, 0.3, and 1.0 ng/mL	5 and 7 years	– Serum antibody concentrations against tetanus and diphtheria toxoids at ages 5 and 7 years	– Maternal PFOS showed negative correlations with diphtheria antibody concentration at age 5 years – PFASs showed negative associations with diphtheria antibody levels, especially at age 7 years

Grandjean and Budtz-Jorgensen [68]	Faroese birth cohort	1997–2000	431	Cohort	Childhood PFOS, PSOA at 5 years	The median of 17.3, and 4.06 ng/mL for PFOS and PFOA, respectively	7 years	Serum antibody concentrations against tetanus and diphtheria toxoids	Benchmark dose levels were about 1.3 ng/mL serum for PFOS and 0.3 ng/mL serum for PFOA at a benchmark response of 5%
Granum et al. [69]	Norway	2007–2008	99	Cohort	Maternal PFOA, PFOS, PFHxS, PFNA	Geometric mean of 1.1, 5.6, 0.3 and 0.3 ng/mL for PFOA, PFOS, PFHxS, and PFNA, respectively	1, 2, and 3 years	– Measles, rubella, tetanus, and influenza type b antibody titers – IgE antibodies – Common colds and other upper respiratory tract infections, otitis media, pneumonia, gastroenteritis, and urinary tract infection – Dry cough, chest tightness, wheeze eczema or itchiness, atopic eczema, allergy, and asthma	– Inverse association between PFASs and anti-rubella antibodies concentrations at age 3 years – Positive association between maternal concentrations of PFOA and PFNA and the number of episodes of common cold for the children, and between PFOA and PFHxS and the number of episodes of gastroenteritis – No associations were found between maternal PFAS concentrations and the allergy- and asthma-related health outcomes
Mogensen et al. [70]	Faroe Islands	1997–2000	464	Cohort	5- and 7-year PFOA, PFOS, PFHxS	Median concentrations, respectively, for PFOA, PFOS, and PFHxS were: 5 years: 4.1, 17.3, and 0.6 ng/mL; 7 years: 4.4, 15.5, and 0.5 ng/mL	7 years	– Tetanus and diphtheria antibodies concentrations	– Childhood exposures, as reflected by both the age-5 and age-7 PFAS measurements showed strong inverse associations between PFAS exposure and antibody concentrations

(continued)

Table 13.3 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Goudarzi et al. [95]	Japan	2003–2009	1558	Cohort	Maternal PFOA, PFOS, PFHxS, PFDA, PFNA, PFUnDA, PFDoDA, and PFTrDA	Median of 2.0, 4.9, 0.3, 0.5, 1.2, 1.4, 0.2, and 0.3 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFUnDA, PFDoDA, and PFTrDA, respectively	First 4 years of life	– Otitis media, pneumonia, respiratory syncytial virus infection, and varicella	– Association of PFOS with increased odds of total infectious diseases – Association of PFHxS with a higher risk of total infectious diseases only among girls
Pennings et al. [72]	Norway	2007–2008	58–73	Cohort	Maternal PFOA, PFOS, PFHxS, PFNA	Geometric mean of 1.1, 5.6, 0.3, and 0.3 ng/mL for PFOA, PFOS, PFHxS, and PFNA, respectively	3 years	– Transcriptomics profiles in neonatal cord blood – Anti-rubella antibody levels at 3 years – Cold episodes until 3 years	– A set of 27 genes found to be associated with PFAS and common cold episodes and a set of 26 genes associated with PFAS exposure and rubella titers
Dalsager et al. [73]	Denmark	2010–2012	359	Cohort	Maternal concentrations of: PFOA, PFOS, PFHxS, PFDA, PFNA	Median concentrations, respectively, for PFOA, PFOS, PFHxS, PFDA, and PFNA were: 1.7, 8.1, 0.3, 0.3, and 0.7 ng/mL, respectively	1–4 years	– Fever, stuffed or runny nose, cough, wheezy or whistling breathing, eye inflammation, ear pain, discharge from ear, feeling unwell, diarrhea, blood in stool, and vomiting	– Association of higher PFOS and PFOA concentrations with higher odds of fever, the number of episodes of co-occurrence of fever and coughing and fever and nasal discharge

Grandjean et al. [74]	Faroe Islands	1997–2000	516	Cohort	7- and 13-year concentration of: PFOA, PFOS, PFHxS, PFDA, PFNA	Median concentrations, respectively, for PFOA, PFOS, PFHxS, PFDA and PFNA were: 7 years: 4.4, 15.3, 0.5, 0.4 and 1.1 ng/mL; 13 years: 2.0, 6.7, 0.4, 0.3, and 0.7 ng/mL	7 and 13 years	– Tetanus and diphtheria antibody concentrations	– Diphtheria antibody concentrations decreased at elevated PFOS, PFNA, and PFDA concentrations at age 7 years
Grandjean et al. [75]	Faroe Islands	2007–2009	275 and 349	Cohort	Maternal, 18 months and 5-year concentration of: PFOA, PFOS, PFHxS, PFDA, PFNA, and modeled POS and PFOA concentrations at 3, 6, and 12 months	Median concentrations, respectively, for PFOA, PFOS, PFHxS, PFDA, and PFNA were: 18 months: 2.8, 7.1, 0.2, 0.3, and 1.0 ng/mL; 5-years: 2.2, 4.7, 0.3, 0.3, and 1.1 ng/mL	5 years	– Diphtheria and tetanus antibody concentrations	– Higher predicted 3-, 6-, and 12-month PFOS and PFOA concentrations were associated with decreased tetanus antibody concentrations – Higher maternal PFOA, PFOS, and PFHxS concentrations were negatively associated with tetanus antibody levels, whereas higher maternal PFOA, PFOS, and PFDA concentrations were negatively associated with diphtheria antibody levels – Higher 18-month PFOA concentrations were negatively associated with tetanus antibody levels – Higher 5 years PFOA concentrations were associated negatively associated with tetanus antibody levels, whereas higher 5-year PFNA and PFDA concentrations were negatively associated with diphtheria antibody levels

(continued)

Table 13.3 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Pilkerton et al. [76]	US NHANES	1999–2000 and 2003–2004	1012 youth and 1387 adults	Cross-sectional	Youth and adult (≥12 years) PFOS and PFOA	The average PFOA, PFOS: 4.8, 25.1 ng/mL for youths.	Youth: 12–18 years Adults: 19–49 years	– Rubella IgG titers	No significant effect of PFASs on immunity in youths
Allergic conditions and biomarkers									
Wang et al. [77]	Taiwan	2004	244	Cohort	Cord blood PFOA, PFOS, PFHxS, PFNA	Median of 1.7, 5.5, 0.04, and 2.3 ng/mL for PFOA, PFOS, PFHxS, and PFNA, respectively	2 years	– IgE concentrations – Atopic dermatitis	– Positive correlation of cord blood PFOA and PFOS with IgE levels only in boys – No association of PFASs with atopic dermatitis
Okada et al. [78]	Japan	2002–2005	231 and 343	Cohort	Maternal PFOA, PFOS	Geometric mean of 1.2 and 5.0 ng/mL for PFOA and PFOS	18 months	– Cord-blood IgE – Infant food allergy, eczema, wheezing, otitis media, chicken pox, bronchitis, RSV disease, rhinitis, pneumonia, skin infection, and other viral infections	– Cord blood IgE levels decreased with high maternal PFOA in females – No associations between maternal PFOS and PFOA levels and food allergy, eczema, wheezing, or otitis

Dong et al. [79]	Taiwan	2009–2010	456 (case-control)	Case-control	Concurrent PFOA, PFOS, PFHxS, PFDA, PFNA, PFBS, PFDoA, PFHpA, PFHxA, and PFTA	For non-asthmatic: median of 0.5, 28.9, 1.3, 1.0, 0.8, 0.5, 2.7, 0.2, 0.2, and 5.2 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFBS, PFDoA, PFHpA, PFHxA, and PFTA, respectively For asthmatics: Median of 1.2, 33.9, 2.5, 1.1, 1.0, 0.5, 3.8, 0.2, 0.2, and 4.1 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFBS, PFDoA, PFHpA, PFHxA, and PFTA, respectively	10–15 years	– Serum IgE – Absolute eosinophil counts (AEC) – Eosinophilic cationic protein concentrations (ECP) – Asthma	– Positive association of PFASs, except PFTA and PFHxA with asthma – Positive association of PFASs, except PFHxA with at least 2 of the 3 biomarkers (IgE, AEC, and ECP) in children with asthma – Positive association of PFOS, PFDA, PFDoA, and PFTA with asthma severity scores
Humblet et al. [80]	US NHANES	1999–2008	1877	Cross-sectional	Concurrent PFOA, PFOS, PFHxS, PFNA	Not presented	12–19 years	– Self-reported lifetime asthma, recent wheezing, and current asthma	– Association of PFOA with higher odds of asthma – Inverse association of PFOS with both asthma and wheezing

(continued)

Table 13.3 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Okada et al. [81]	Japan	2003–2009	2063	Cohort	Maternal PFOA, PFOS, PFHxS, PFDA, PFNA, PFUnDA, PFDoDA, and PFTrDA	Geometric mean of 2.1, 5.0, 0.3, 0.5, 1.2, 1.3, 0.2, and 0.3 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFUnDA, PFDoDA, and PFTrDA, respectively	12 and 24 months	– Allergic diseases, eczema, wheezing, and allergic rhinoconjunctivitis symptoms	– Lower odds of eczema with higher maternal PFTrDA levels only in female infants
Osuna et al. [82]	Faroe Islands	1986–1987	38	Cohort	Cord blood and 7-year PFOA and PFOS	Median concentrations, respectively, for PFOA and PFOS were: cord blood: 0.7, 3.1 ng/mL; 7-years: 4.3, 27.0 ng/mL	7 years	– IgM and IgG autoantibodies specific to neural and nonneural antigens at age 7	– Inverse association of PFOS with anti-actin IgG
Ashley-Martin et al. [83]	Canada	2008–2011	1258	Cross-sectional	1st-trimester maternal PFOA, PFOS, PFHxS	Geometric mean of 1.7, 4.6, and 1.0 ng/mL for PFOA, PFOS, and PFHxS, respectively	At birth	– Umbilical cord blood levels of IgE, TSLP, and IL-33	– No association of PFASs with immunotoxic effects that manifest as increased odds of elevated levels of IgE, TSLP, or IL-33

Smit et al. [84]	Greenland and Ukraine	2002–2004	1024	Cohort	Maternal PFOA, PFOS, PFHxS, PFDA, PFNA PFHpA, PFUnDA, and PFDoDA	Ukraine: Geometric mean of 1.0, 4.9, 1.5, 0.2, 0.6, 0.03, 0.2, and 0.04 ng/mL PFOA, PFOS, PFHxS, PFDA, PFNA, PFHpA, PFUnDA, and PFDoDA, respectively Ukraine: geometric mean of 1.8, 20.6, 2.1, 0.4, 0.7, 0.05, 0.7, and 0.1 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFHpA, PFUnDA, and PFDoDA, respectively	5–9 years	– Asthma, eczema, and wheeze	– Inverse association of PFOA with current wheeze in Ukrainian children
Buser and Scinicariello [85]	US NHANES	2005–2006 and 2007–2010		Cross-sectional	Concurrent PFOA, PFOS, PFHxS, PFNA	– Geometric mean serum PFOA, PFNA, PFOS, and PFHxS were: – NHANES 2005–2006: 3.6, 0.9, 15.0, and 2.1 ng/mL, respectively NHANES 2007–2010: 3.3, 1.1, 8.7, and 2.2 ng/mL, respectively	12–19 years	– Serum food-specific IgE levels (egg, milk, peanuts, and shrimp) (2005–2006) – Self-report of food allergies (2007–2010)	– Inverse association of PFNA with food sensitization when using IgE levels – Serum PFOA, PFOS, and PFHxS were statistically significantly associated with higher odds to have self-reported food allergies
Goudarzi et al. [71]	Japan	2003–2009	1558	Cohort	Maternal PFOA, PFOS, PFHxS, PFDA, PFNA, PFUnDA, PFDoDA, and PFTrDA	Median of 2.0, 4.9, 0.3, 0.5, 1.2, 1.4, 0.2, and 0.3 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFUnDA, PFDoDA, and PFTrDA, respectively	4 years	– Allergic conditions including eczema, wheeze and rhinoconjunctivits	– Inverse association of PFDoDA and PFTrDA (long-chain PFASs) with total allergic diseases (presence of at least one allergic condition) and eczema

(continued)

Table 13.3 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Stein et al. [86]	US NHANES	1999–2006	1191 and 640	Cross-sectional	Concurrent PFOA, PFOS, PFHxS, PFNA	Geometric mean of 4.1, 20.8, 2.5, and 0.8 ng/mL for PFOA, PFOS, PFHxS, and PFNA, respectively	12–19 years	– Measles, mumps, and rubella antibody titers – Asthma, wheeze, allergy, and rhinitis	– Association of higher PFOS concentrations among seropositive children with decreased rubella and mump antibody concentrations – No adverse association between PFAS exposure and current allergic conditions, including asthma
Zhu et al. [87]	Taiwan	2009–2010	456 (case-control)	Case-control	Concurrent PFOA, PFOS, PFHxS, PFDA, PFNA, PFBS, PFDoA, PFHpA, PFHxA, and PFTA	For non-asthmatic: Median of 0.5, 28.9, 1.3, 1.0, 0.8, 0.5, 2.7, 0.2, 0.2, and 5.2 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFBS, PFDoA, PFHpA, PFHxA, and PFTA, respectively For asthmatics: median of 1.2, 33.9, 2.5, 1.1, 1.0, 0.5, 3.8, 0.2, 0.2, and 4.1 ng/mL for PFOA, PFOS, PFHxS, PFDA, PFNA, PFBS, PFDoA, PFHpA, PFHxA, and PFTA, respectively	10–15 years	– TH1 [interferon (IFN)-γ, interleukin (IL)-2] and TH2 (IL-4 and IL-5) cytokines	– Serum PFOA, PFOS, PFBS, and PFNA were associated positively with TH2 cytokines, whereas PFDA and PFNA were negatively associated with TH1 cytokines among male asthmatics

Oulhote et al. [88]	Faroe Islands	2007–2009	56	Cohort	Maternal, 18-month and 5-year concentration of: PFOA, PFOS, PFHxS, PFDA, PFNA	Geometric mean concentrations, respectively, for PFOA, PFOS, PFHxS, PFDA, and PFNA were: maternal: 1.5, 9.1, 0.2, 0.3, and 0.8 ng/mL; 18 months: 3.6, 8.3, 0.2, 0.3, and 1.2 ng/mL; 5 years: 2.6, 5.1, 0.4, 0.4, and 1.4 ng/mL	5 years	– WBCs: neutrophils, eosinophils, lymphocytes, and monocytes – T cell (CD3), T-helper cells (CD4), T-cytotoxic cells (CD8), B-lymphocytes (CD19), NK (CD16/56) cells, and CD4+ recent thymic emigrants (CD4-RTE)	– 5-year latent function combining PFAS concentrations were associated with higher basophil counts
Qin et al. [89]	Taiwan	2009–2010	Asthma 132 Non-asthma 168	Case-control	8 PFASs in children's serum	The median of children with asthma is PFOS: 31.51, PFOA: 1.02, PFBS: 0.48, PFDA: 1.13, PFHxA: 0.20, PFHxS: 2.38, PFNA: 1.00, PFTA: 2.65, and without asthma is PFOS: 28.83, PFOA: 0.50, PFBS: 0.48, PFDA: 0.93, PFHxA: 0.18, PFHxS: 1.07, PFNA: 0.80, PFTA: 4.52 ng/mL	10–15 years	– Asthma – Lung function: forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), peak expiratory flow rate (PEF), and forced expiratory flow 25–75% (FEF25–75), total lung capacity (TLC) and residual volume (RV)	Association of serum PFASs with decreased lung function among children with asthma
Timmermann et al. [90]	Faroe Islands	1997–2000	581 at 5 years and 491 at 13 years	Cohort	Maternal-, 5- and 13-year concentration of: PFOA, PFOS, PFHxS, PFDA, PFNA	Median concentrations, respectively, for PFOA, PFOS, PFHxS, PFDA, and PFNA were: maternal: 3.3, 27.4, 4.5, 0.3, and 0.6 ng/mL; 5-years: 4.0, 16.8, 0.6, 0.3, and 1.0 ng/mL; 13-years: 2.0, 6.7, 0.4, 0.3, and 0.7 ng/mL	5 years and 13 years	– Total IgE, asthma, allergy – Positive skin prick test – Allergic rhinoconjunctivitis and atopic eczema	– Association of PFASs at age 5 years with increased odds of asthma at ages 5 and 13 only in a group of MMR-unvaccinated children – No association of prenatal PFAS exposure with childhood asthma or allergic diseases

(continued)

Table 13.3 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Reported concentrations of PFASs	Age of outcome assessment	Outcome measures	Primary findings
Zhou et al. [91]	Taiwan	2009–2010	456 (case-control)	Case-control	PFOA, PFOS, PFHxS, PFDA, PFNA, PFBS, PFDoA, PFHpA, PFHxA, and PFTA in children's serum	The median of boys and girls with asthma of PFOS: 36.9, 28.2, PFOA: 1.3, 0.8, PFBS: 0.5, 0.5, PFDA: 1.2, 1.1, PFDoA: 4.3, 2.9, PFHxA: 0.2, 0.2, PFHxS: 2.6, 2.5, PFNA: 1.0, 0.9, PFTA: 3.9, 5.9 ng/mL, and without asthma of PFOS: 29.9, 28.8, PFOA: 0.5, 0.5, PFBS: 0.5, 0.5, PFDA: 0.9, 1.0, PFDoA: 2.4, 3.1, PFHxA: 0.2, 0.2, PFHxS: 1.4, 1.2, PFNA: 0.8, 0.9, PFTA: 6.0, 4.5 ng/mL	10–15 years	Asthma and urinary CC16	– Asthmatic participants had significantly higher serum PFAS concentrations overall than the healthy controls – Inverse association of PFASs with CC16 levels especially among males
Zhou et al. [92]	Taiwan	2009–2010	456 (case-control)	Case-control	PFOA, PFOS, PFHxS, PFDA, and PFNA in children's serum	The median of children with asthma of PFOS: 33.94, PFOA: 1.16, PFDA: 1.14, PFHxS: 2.47, PFNA: 1.00, and without asthma of PFOS: 28.91, PFOA: 0.52, PFDA: 0.95, PFHxS: 1.32, PFNA: 0.83 ng/mL	10–15 years	– Interaction between PFASs and reproductive hormones on asthma	– Among asthmatics, positive association between PFASs and estradiol levels and negative association between PFASs and testosterone levels

Chen et al. [93]	China	2012–2015	687	Cohort	10 PFASs in cord blood	The median of PFOS: 2.48, PFOA: 6.98, PFNA: 0.64, PFDA: 0.36, PFUA: 0.4, PFDoA: 0.09, PFHxS: 0.16, PFBS: 0.05 ng/mL	Up to 24 months	– Childhood atopic dermatitis at 6, 12, and 24 months	Association of PFOA, PFDA, PFDoA, and PFHxS with increased risk of childhood atopic dermatitis in female children during the first 24 months of life
Impinen et al. [94]	Norway	1992–1993, 1994–1995, 2002–2003	641	Cohort	19 PFASs in cord blood	Median of 5.2, 1.6, 0.4, 0.2, 0.2, and 0.1 ng/mL for PFOS, PFOA, PFOSA, PFHxS PFNA, and PFUnDA, respectively	2 and 10 years	– Obstructive airway disease (wheeze by 10 years; asthma by 2 and 10 years; reduced lung function at birth; allergic rhinitis by 10 years) – Atopic dermatitis by 2 and 10 years – Allergic sensitization by 10 years – Episodes of common respiratory tract infections (common cold by 2 years, lower respiratory tract infections by 10 years)	– Although prenatal exposure to PFASs was not associated with atopic or lung manifestations by 10 years of age, several PFASs were associated with an increased number of respiratory tract infections in the first 10 years of life

Five epidemiological studies have assessed infections as outcomes, and most studies found an association of PFASs with increased risk of childhood infections. In the Hokkaido study, no associations were observed between prenatal PFAS exposures and mother-reported infectious diseases, including otitis media, chicken pox, skin infections, pneumonia, bronchitis, respiratory syncytial virus infection, and other viral infections among Japanese infants during the first 18 months of life [78]. In a Danish study, higher prenatal PFOA and PFOS concentrations were associated with co-occurrence of fever and coughing and fever and nasal discharge among children at ages 1–4 years [73]. In a sub-cohort of the Norwegian Mother and Child Cohort Study (MoBa), prenatal PFOA and PFNA concentrations were associated with the number of episodes of common cold among children up to age 3; also PFHxS and PFOA were associated with an increased number of gastroenteritis episodes in children [69]. Another Norwegian study examined the association of cord blood PFASs and episodes of common upper (common cold by 2 years) and lower respiratory tract infections (by 10 years). The authors reported an association of PFOS, PFOA, PFOSA, PFNA, and PFUnDA with increased lower respiratory tract infections. Additionally, the positive association between PFUnDA and common colds episodes at 2 years was reported [94]. The Hokkaido Study also linked higher maternal PFAS concentrations with an increased prevalence of infectious diseases in children, including otitis media, pneumonia, varicella, and respiratory virus infection, in children up to 4 years of age, especially in girls [95]. However, a study from DNBC showed no convincing associations between prenatal PFOS and PFOA and risk of hospitalizations due to infections classified according to ICD-10 among children with an average age of 8.2 years [66]. Apart from the latter study that explored hospital admission data up to mid-childhood in regard to prenatal exposure only, the studies available suggest that increased incidence of common childhood infections is a likely outcome of elevated PFAS exposures. This conclusion is in excellent agreement with the separate finding of lowered antibody responses following vaccinations.

13.4.2 Allergic Conditions Such as Wheeze, Rhinitis, Eczema, and Biomarkers

Nineteen epidemiological studies assessed allergic conditions/diseases, and biomarkers. About half of the studies were case-control or cross-sectional in nature. Most of the studies examined allergic conditions based on maternal reports. IgE was the most examined biomarker among biomarker candidates.

13.4.2.1 Allergic Conditions and IgE

In Hokkaido study, no association was observed between prenatal exposure to PFOS and PFOA with allergic diseases including food allergy, wheezing, and eczema during the first 18 months of life. Also, an inverse association was observed

between prenatal PFOA exposure and cord blood IgE among female infants [78]. A Canadian study reported no association between in utero PFAS exposures and cord blood IgE, thymic stromal lymphopoietin (TSLP), and interleukin-33 (IL-33) levels [83]. IN TPBC study, no association was observed between cord blood PFASs and atopic dermatitis defined by the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire; however, PFASs were positively associated with cord blood IgE levels [77]. A small Norwegian study reported no associations between prenatal exposure to PFAS concentrations and pulmonary symptoms, wheeze/asthma, and eczema/itchiness among children up to age 3 [69]. Another study from Norway did not observe any convincing association of cord blood PFAS concentrations with allergy- and asthma-related outcomes at age 2 and 10 years [94].

In China, maternal PFHxS, PFOA, PFNA, PFDA, and PFDoA exposure levels were associated with the risk of doctor-diagnosed atopic dermatitis in female infants by age 2 years [93]. A study examined pre- and postnatal (at ages 5 and 13 years) PFAS exposures and collected parents' reports on allergic conditions at ages 5 and 13. The researchers found that prenatal PFAS exposure was not associated with childhood asthma, rhinoconjunctivitis, and eczema regardless of MMR vaccination status. Among children with no MMR vaccination, postnatal PFASs at age 5 were positively associated with the risk of asthma at ages 5 and 13. However, reversed associations among MMR-vaccinated children were found. Also, PFAS exposure at age 5 did not show associations with total IgE at age 7, and positive skin prick test at age 13 [90].

The Hokkaido birth cohort also reported the inverse association of prenatal PFAS exposures with the risk of eczema and total allergic diseases (presence of one of the three allergic conditions including eczema, wheeze, and rhinoconjunctivitis) using ISAAC questionnaires after sex stratification; the associations were significant among female infants [81]. In the same cohort, inverse associations of prenatal PFAS concentrations, mainly long-chain PFASs such as PFDoDa and PFTrDA with an eczema and total allergic diseases among children at age 4, were found [71]. The INUENDO birth cohort, examining several environmental chemicals among children from Greenland and Ukraine, also did not find convincing associations between prenatal PFASs and allergic conditions among 5- to 9-year-old children [84].

An NHANES-based study with cross-sectional design among children aged 12–19 found that childhood PFOA concentrations were associated with increased risk of asthma diagnosis, while PFOS was inversely associated with the risk of wheeze and asthma [80]. Another report from NHANES did not observe associations between childhood PFASs and wheeze, rhinitis, and asthma in the same age range [86]. However, they found a positive association between PFASs and allergen-specific-IgE levels in those children [85]. A case-control study in Taiwan found an association between PFOA and PFDoDA with increased risk of asthma among children in 10–15 years of age recruited in the Genetic and Biomarkers study for Childhood Asthma (GBCA). Also, the positive association of PFASs with serum IgE concentrations, eosinophilic cationic protein concentrations, absolute eosinophil counts, and asthma severity scores was reported among asth-

matics [79]. In the same study and participants, asthmatic cases had significantly higher serum PFAS concentrations overall than the healthy controls. Also, PFASs showed a negative association with urinary levels of 16 kDa club cell secretory protein (CC16) which is a major anti-inflammatory airway protein [91]. Another report from the same study population showed that reproductive hormones may amplify the relationship between PFASs and asthma among adolescents [92]. Moreover, another cross-sectional case-control study of GBCA found that serum PFAS levels among asthmatic children, but not healthy controls, were inversely associated with pulmonary function measurements such as forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) among children with asthma [89]. Overall, these reports indicate that PFAS may modulate immune functions and potentially increase the risk of allergic disease, but several cofactors, such as family history and allergen exposure, may play a role and would be difficult to fully adjust for.

13.4.2.2 Other Immune Biomarkers

A small study from the Faroe Islands which measured pre- and postnatal (18 months and 5 years) PFAS levels found the positive association of 5-year PFAS concentrations with basophil counts, but not eosinophil, neutrophil, and monocyte count at the same. Also, no associations were observed for lymphocyte subpopulations such as B cells, natural killer cells, CD3+, CD4+, and CD8+ T cells [88]. In contrast, A case-control Taiwanese study reported that concurrent PFAS concentration is associated with reduced T-helper 1 cytokines (interferon-γ and IL-2) and increased T-helper 2 cytokines (IL-4 and IL-5) among male asthmatic subjects suggesting that PFASs may dysregulate TH1 and Th2 balance by gender differences [87]. Lastly, a very small study ($n = 38$) that investigated IgM and IgG autoantibodies specific to neural and nonneural antigens with small sample size reported an inverse association between 7-year anti-actin IgG antibodies and in utero PFOS exposure among Faroese children [82].

Overall, several epidemiological studies reported inconsistent finding on the association of perinatal PFAS exposures and allergic conditions such as wheeze, rhinitis, eczema, and asthma. However, most of the previous studies suggested an increased risk of infectious diseases and reduced vaccine response in children prenatally exposed to PFASs. Findings on allergic diseases are inconsistent. Most of the studies on allergic conditions are based on subjective assessment of allergic symptoms and few studies have doctor-diagnosed allergic diseases and asthma. Additionally, different methodological designs, timing of exposure assessment, diet and socioeconomic status, and control for confounding make a comparison of studies difficult and establishing a causal relationship between PFASs and immune system endpoints needs more longitudinal epidemiological studies across several populations with valid and reliable outcome measures. However, differences in

studied endpoints, windows of exposure, age at outcomes evaluation, and potential for outcome misclassification may explain some of the inconsistency in findings.

13.5 Endocrine System

We found 23 epidemiological studies regarding the association of PFAS with endocrine system endpoints including thyroid and steroid hormones from birth to adolescence (Table 13.4).

13.5.1 Thyroid Hormones

A Korean study with small sample size measured PFASs and thyroid hormones in maternal and cord blood samples and found that most associations were null except inverse associations between maternal PFOS and fetal T3, and maternal PFTrDA and fetal T4 and T3 [97]. A Norwegian study examined the association of PFASs with maternal thyroid hormones and thyroid-binding proteins measured during the second trimester, 3 days and 6 weeks after delivery. This study reported a positive association between PFOS and thyroid-stimulating hormone (TSH). Also, PFDA and PFUnDA were inversely associated with T3 and FT3, respectively [100]. Another study from Korea with retrospective design showed a positive association between prenatal PFPeA and cord blood T4 levels. Among girl infants, the association between PFHxS with increased T3, and PFPeA with T4, while PFNA with the reduced TSH levels, was observed [101]. Taiwan Maternal and Infant Cohort Study (TMICS), reported a positive association of maternal PFOS and TSH. Also, several PFASs were associated with lower free T4 and total T4 among pregnant women. In addition, some maternal PFASs were associated with lower cord total T3 and T4 levels [99]. A Canadian study found that maternal PFASs were associated with increased TSH, and weakly inversely associated with fT4 in the subset of pregnant women with high TPOAb [98].

Project Viva in the USA reported a negative association of some of prenatal PFASs with free T4 index levels, but not maternal T4 levels with cross-sectional design. PFOA, PFOS, and PFNA levels were associated with reduced TSH in TPOAb-positive women only; however, no associations were observed in TPOAb-negative women and the full population. Also, an association of prenatal PFOA, PFOS, and PFHxS concentrations with decreased T4 levels was found only among male infants [103]. A Canadian study examined thyroid hormones and TPOAb in maternal plasma collected in each trimester and 3 months postpartum, and found an association between early-pregnancy PFASs with sulfhydryl group (PFHxS and branched PFOS) and increased TSH and reduced FT4 during and after pregnancy [106]. In Hokkaido study, maternal PFOS, but not

Table 13.4 Results of epidemiological studies assessing associations of developmental exposure to PFASs with endocrine system outcomes

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Concentrations of PFASs	Outcome measures	Primary findings
Thyroid hormones								
Kim et al. [97]	South Korea	2008–2009	35–44 mother-infant pairs	Cohort and cross-sectional	13 PFASs from serum of pregnant women, cord blood	The median (ng/mL) of prenatal: PFHxS 0.5, PFOS 2.9, PFOA 1.4, PFNA 0.4, PFDA 0.3, PFUnDA 0.6, PFTrDA 0.2 Cord blood: PFHxS 0.3, PFOS 1.2, PFOA 1.1, PFNA 0.4, PFDA 0.2, PFUnDA 0.5, PFTrDA 0.1	Cord blood total T4, T3 and TSH	Negative correlations between maternal PFOS and fetal T3, and maternal PFTrDA and fetal T4 and T3
Webster et al. [98]	Canada	2006–2008	152	Cross-sectional	4 maternal serum PFASs	The median (ng/mL) of: PFHxS 1.0, PFOS 4.8, PFOA 1.7, PFNA 0.6	Repeated measures of maternal fT4, TT4, and TSH and TPOAb	– PFASs were associated with a hypothyroid effect in pregnant women with high TPOAb. PFASs were associated with low fT4 and high TSH in these women – No associations were found in women with normal TPOAb

Wang et al. [99]	Taiwan	2000–2001	Pregnant women ($n = 285$), neonates ($n = 116$)	Cross-sectional	9 maternal serum PFASs	The median (ng/mL) of: PFHxS 0.8, PFOS 12.7, PFOA 2.4, PFNA 1.5, PFDeA 0.4, PFUnDA 3.2, PFDoDA 0.3	Maternal and FT4, TT4, TT3, and TSH	– Positive association of maternal PFOS and TSH levels – Association of PFNA, PFUnDA, and PFDoDA with lower maternal FT4 and TT4 levels – Maternal PFNA, PFUnDA, and PFDoDA were associated with lower cord TT3 and TT4 levels, and PFDeA was associated with lower cord TT3
Berg et al. [100]	Norway	2007–2009	375	Cohort and cross-sectional	7 detectable PFASs in maternal serum	The median (ng/mL) of PFHxS 0.4, PFOS 8.0, PFOA 1.5, PFNA 0.5, PFDA 0.2, PFUnDA 0.2	Maternal THs, thyroid-binding proteins (TH-BP), thyroxin-binding capacity and anti-TPO, at three visits (during the second trimester, 3 days and 6 weeks after delivery)	– Positive association of maternal PFOS with TSH – Inverse association of maternal PFDA and PFUnDA with T3 and FT3, respectively
Shah-Kulkarni et al. [101]	South Korea	2006–2010	279	Retrospective cohort	Cord blood	The median (ng/mL) of: PFHxS 0.3, PFOS 0.6, PFOA 0.9, PFTrDA 0.4, PFUnDA 0.2, PFPeA 0.2, PFNA 0.2, PFDA 0.1, PFDoDA 0.08, and PFTeDA 0.06	Cord blood thyroid hormone levels (T3, T4, and TSH)	– Positive association of PFPeA with T4 levels – Positive association of PFHxS with T3 concentration, PFPeA with the T4 concentration in females – Inverse association between PFNA and TSH levels in females

(continued)

Table 13.4 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Concentrations of PFASs	Outcome measures	Primary findings
Kato et al. [102]	Japan	2002–2005	392	Cohort and cross-sectional	2 maternal serum PFASs (during and after pregnancy)	The median (ng/mL) of: PFOS 5.2, and PFOA 1.0	TSH and FT4 levels of mothers (early pregnancy), and infants (between 4 and 7 days of age)	Maternal PFOS levels, but not PFOA, were inversely correlated with maternal serum TSH and positively associated with infant serum TSH
Peterson et al. [103]	USA (Project Viva)	1999–2002	732 (maternal samples) 480 (neonatal samples)	Cohort and cross-sectional	6 maternal early pregnancy plasma PFASs	The median (ng/mL) of: PFHxS 2.4, PFOS 24.0, PFOA 5.6, PFNA 0.6, EtFOSAA 1.1, MeFOSAA 1.8	– Maternal: T4, free T4 index (FT4I), (TSH) in plasma samples collected at early pregnancy – Neonatal: T4 levels from postpartum heel sticks	– Inverse associations of PFOA, PFHxS, and MeFOSAA concentrations with FT4I levels (not T4) in maternal – Inverse association of PFOA, PFOS with TSH in TPOAb-positive women only – Association of prenatal PFOA, PFOS, and PFHxS with lower postpartum T4 levels in male neonates
Dufour et al. [104]	Belgium	2013–2016	221	Cross-sectional	7 PFASs in cord blood (4 PFASs with detection rate more than 350%)	The median (ng/mL) of: PFHxS 0.1, PFOS 0.7, PFOA 0.6, PFNA 0.1	Cord blood TSH	Inverse association of PFNA with TSH level in male infants

Aimuzi et al. [105]	China	2012–2013	568	Cross-sectional	10 PFAS in cord plasma	The median (ng/mL) of: PFHxS 0.1, PFOS 2.5, PFOA 7.5, PFNA 0.6, PFDA 0.4, PFUnDA 0.4, PFDoDA 0.1, PFBS 0.05	FT3, FT4, TSH levels in prelabor caesarean deliveries	– Inverse association of PFOS, PFNA, PFDA, PFUA, and PFDoA with TSH – Association of PFDoA with increased FT4 and decreased FT3 – Association of PFOS with increased FT3
Reardon et al. [106]	Canada	2009–2012	494	Cohort and cross-sectional	8 PFASs (and branched isoforms) in second trimester maternal plasma/serum	The median (ng/mL) of PFHpA 0.08, PFHxS 1.0, PFOS 4.7, PFOA 2.1, PFNA 0.7, PFDA 0.2, PFUnDA and PFDoDA 0.06	FT4, free FT3, TSH and TPOAb in maternal plasma collected in each trimester and 3 months postpartum	Generally, associations were strongest in early pregnancy, and influenced by mercury co-exposure and thyroid peroxidase antibodies – Branched PFOS isomers were positively associated with TSH in a dose-dependent manner that were strongest in early pregnancy (first trimester) and weakened over subsequent trimesters – Inverse association of PFHxS with FT4

(continued)

Table 13.4 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Concentrations of PFASs	Outcome measures	Primary findings
Steroid hormones								
(a) Infancy								
Itoh et al. [107]	Japan	2002–2005	189	Cohort	2 PFASs in maternal serum	The median (ng/mL) of PFOS 5.2, PFOA 1.4	Cord blood E2, P, T, FSH, LH, inhibin B, PRL, insulin-like factor 3, SHBG	– In male infants: Association of PFOS with increased E2 and reduced T/E2, P, and inhibin B. Association of PFOA with increased inhibin B – In female infants: Association of PFOS with reduced P and PRL
Goudarzi et al. [108]	Japan	2002–2005	185	Cohort	2 PFASs in maternal serum	The median (ng/mL) of PFOS 5.2, PFOA 1.4	Cord blood glucocorticoids (cortisol and cortisone), and androgenic hormones (DHEA and androstenedione)	– Association of PFOS, but not PFOA, with reduced cortisol and cortisone – Association of PFOA and PFOA with increased and decreased DHEA, respectively

Wang et al. [109]	China	2013	424	Cross-sectional	2 PFASs in cord blood	The median (ng/mL) of: PFOS 0.6, PFOA 2.0	Cord blood E1, E2, and E3	– Positive association of PFOS with E1 and E3, but inverse association with E2 – Positive association of PFOA with E1
Yao et al. [110]	China	2010–2013	116–351	Cross-sectional	10 PFASs in cord blood	The median (ng/mL) of PFHxS 0.3, PFOS 1.4, PFOA 34.6, PFBS 0.2, PFNA 0.4, PFDA 0.2, PFDoDA 0.1, PFHpA 0.1, PFOSA 0.1, PFUnDA 0.1	Cord blood E2, T, P450arom, 3β-HSD1, and 17β-HSD1	– Positive associations of PFOA, PFHxS with E2 levels, PFOS, PFUA, PFNA with T levels, and PFOS, PFUA with T/E2 ratio – Association of PFUA, PFNA, PFDA, PFHxS, and ∑PFASs with higher P450arom levels – Association of PFHxS with increased 3β-HSD1 and 17β-HSD1 levels
(b) Childhood, adolescence, and adulthood								
Maisonet et al. [111]	UK	1991–1992	72	Cohort	4 PFASs in maternal serum samples	The median (ng/mL) of PFHxS 1.6, PFOS 19.2, PFOA 3.6, PFNA 0.5	Serum total T and SHBG only among female offspring at 15 years of age	– Prenatal exposure to PFOA, PFOS, and PFHxS was associated with higher total T concentrations – No associations between PFASs and SHBG

(continued)

Table 13.4 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Concentrations of PFASs	Outcome measures	Primary findings
Kristensen et al. [112]	Denmark	1988–1989	343	Cohort	2 PFASs in maternal serum	The median (ng/mL) of PFOS 21.1, PFOA 3.6	Age of menarche, menstrual cycle length, follicle number, and reproductive hormones (FSH, LH, AMH, E2, total T, SHBG, FAI) of female offspring at around 20 years of age	– Association between prenatal exposure to PFOA, but not PFOS, and later age of menarche – No effects of PFASs on menstrual cycle length, follicle number, and reproductive hormone concentrations
Joensen et al. [113]	Denmark	2003	105 (men)	Cross-sectional	10 serum PFASs	The median (ng/mL) of: PFHxS 6.6, PFOS 24.5, PFOA 4.9, PFNA 0.8, PFDA 0.9, PFHpA 0.2, PFOSA 0.06, PFUnDA 0.1, PFDoDA 0.08	T, E2, SHBG, LH, FSH, and inhibin B, FAI, semen analysis (volume, count, motility, and morphology) among young men with median age of 19 years	Men with high combined levels of PFOS and PFOA had a median of 6.2 million normal spermatozoa Nonsignificant trends with regard to lower sperm concentration, lower total sperm counts, and altered pituitary–gonadal hormones among men with high PFOS–PFOA levels

Joensen et al. [114]	Denmark	2008–2009	247 (young healthy men)	Cross-sectional	14 serum PFASs	The median (ng/mL) of: PFHxS 0.6, PFOS 7.8, PFOA 3.0, PFNA 1.0, PFDA0.3, PFHpS 0.2	– Serum total T, E2, SHBG, LH, FSH, and inhibin-B – Semen assessment (weight, sperm concentration, count, motility, morphology)	– Inverse association of PFOS levels with T, calculated free testosterone (FT), FAI, and ratios of T/LH, FAI/LH, and FT/LH – No association of other PFASs and reproductive hormones – No association between PFASs and semen quality
Barret et al. [115]	Norway	2002–2003	178 (young healthy women)	Cross-sectional	10 serum PFASs	The median (nulliparous; parous women; ng/mL) of: PFOA (3.3; 2.0), PFOS (14.7; 12.6), PFNA (0.6; 0.5), PFDA (0.2; 0.2), PFUnDA (0.3; 0.3), PFHxS (1.0; 0.7), PFOSA (0.2; 0.1), PFHpS (0.1; 0.1), PFHpA (0.09; 0.09), and PFDoDA (0.07; 0.08)	Saliva E2 and P in healthy, naturally cycling women between 25 and 35 years of age	– Association of PFOS and PFOA with reduced E2 and P – No association of other PFASs with outcomes
Vested et al. [116]	Denmark	1988–1989	169	Cohort	2 PFASs in maternal serum	The median (ng/mL) of: PFOS 21.2, PFOA 3.8	Semen quality, testicular volume, and reproductive hormones (T, E2, SHBG, LH, FSH, and inhibin B, FAI) in male offspring (19–21 years)	– Prenatal PFOA, but not PFOS, was associated with lower sperm concentration and total sperm count and with higher levels of LH and FSH

(continued)

Table 13.4 (continued)

Study	Country	Year of enrollment	No. assessed	Study design	Exposure	Concentrations of PFASs	Outcome measures	Primary findings
Zhang et al. [50, 117]	China	2013–2016	120 healthy controls 120 with POI	Case-control	9 PFASs in plasma samples of women	The median (ng/mL) of cases vs. controls: PFHxS 0.38 vs. 0.29, PFOS 8.1 vs. 6.0, PFOA 11.1 vs. 8.3	Risks of POI, reproductive hormones (FSH, LH, E2, T, PRL) among women aged 20–40	– Association of PFOA, PFOS, and PFHxS with increased risks of POI
Petersen et al. [103]	Faeroe islands	2007–2009	263	Cross-sectional	5 PFASs in serum samples of men	The median (ng/mL) of: PFOS 19.5, PFOA 2.8	Semen quality (concentration, total count, volume, morphology and motility) or serum reproductive (FSH, LH, SHBG, T, E2, and inhibin B) in Faroese men (24–26 years)	Positive association of PFOS with SHBG and LH
Heffernan et al. [118]	UK	2015	30 with PCOS 29 controls	Case-control	13 PFASs in serum and follicular fluids	The median (ng/mL) of in serum: PFHxS 0.9, PFOS 3.1, PFOA 2.4, PFNA 0.5	Serum T, E2, FAI, SHBG in cases and controls undergoing fertility treatment	– Serum PFOS was higher in PCOS cases than controls – Association of PFOA, PFHxS, PFNA, with T among controls but not PCOS cases

AMH anti-Mullerian hormone, *DHEA* dehydroepiandrosterone, *FAI* free androgen index, *FT4I* free, *T4* index, *E1* estrone, *E2* estradiol, *E3* estriol, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *P* progesterone, *PCOS* polycystic ovarian syndrome, *POI* premature ovarian insufficiency, *PRL* prolactin, *SHBG* steroid hormone-binding globulin, *T* testosterone, *TSH* thyroid-stimulating hormone
3β-HSD1 human 3 beta hydroxysteroid dehydrogenase type 1, *17β-HSD1* human 17 beta hydroxysteroid dehydrogenase type 1, *P450arom* CYP19A1, P450-family19-subfamily A polypeptide 1

PFOA, was inversely associated with maternal TSH, and was associated with increased serum TSH among Japanese infants [102]. A study in Shanghai found a concurrent inverse association between cord blood PFASs and thyroid-stimulating hormone (TSH) concentrations. The associations between PFASs and free T4 and T3 were inconsistent [105]. A Belgian study found an association between cord blood PFNA concentration and reduced TSH in male newborns. PFOA and PFOS higher levels were associated with increased risk of hypothyroid history in mothers [104].

Lastly, ATSDR [1] concluded that there is an increased risk of thyroid disease at elevated exposures to PFOS and PFOA, a conclusion that is in agreement with a recent systematic review [120].

13.5.2 Steroid Hormones and Reproductive Function

In the Hokkaido study on male infants, prenatal PFOS concentrations were significantly associated with increased estradiol (E2) and decreased testosterone (T)/E2, progesterone, and inhibin B in cord blood among male infants. Also, a positive association was observed between prenatal PFOA and cord blood inhibin B levels. Among the female infants, an inverse association between PFOS and progesterone and PRL levels was found [107]. In the same cohort and almost the same participants, cord blood glucocorticoid and androgenic hormone levels were investigated, and a dose-response inverse association of prenatal PFOS concentrations with infant cortisol with cortisone levels was observed. Also, cord blood DHEA levels, but not androstenedione, were positively associated with prenatal PFOS and negatively with PFOA in a dose-response manner [108].

A cross-sectional Chinese study reported positive associations between cord blood PFASs and T and T/E2 ratio. Also, PFASs were positively associated with P450arom, 3β-HSD1, and 17β-HSD1 levels in placental tissue samples suggesting a potential mediatory role of the placental steroidogenic enzyme in PFAS and steroid relationship [110]. Another Chinese cross-sectional study showed an association of PFOS and PFOA with estrone (E1), E2, and estriol (E3) in different directions [109]. A Danish study reported that prenatal exposure to PFOA, but not PFOS, was associated with lower sperm concentration and total sperm count, and increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels among male offspring aged 19–21 years [116]. A study among 15-year-old female participants in the UK found that prenatal exposure to PFASs was associated with increased total testosterone levels but not sex hormone-binding globulin (SHBG) levels [111]. A cross-sectional study from DNBC among female participants at age 20 reported that prenatal exposure to PFOA, not PFOS, was associated with delay of menarche. However, PFASs did not have any association with menstrual cycle length, follicle number, and reproductive hormone concentrations [112]. Another cross-sectional

study among young Danish men found an association between serum PFASs and decreased numbers of normal spermatozoa. Additionally, PFASs showed some tendencies to be associated with reduced sperm concentration, total sperm count, and sperm motility, however [113]. The same group in another population of young healthy men with a median age of 19 years observed that concurrent serum PFOS concentration was associated with reduced total and free testosterone levels, but not with semen quality in Denmark. Also, PFASs did not show any association with reproductive hormones [114]. A Norwegian study among healthy and naturally cycling women with age range between 25 and 35 years found an inverse association between PFOS concentrations with E2 and progesterone only in nulliparous women [115].

A cross-sectional study among Faroese young men observed a positive association between serum PFOS concentrations and higher levels of SHBG and LH. However, PFOS and PFOA did not show association with any sperm motility, morphology, and concentration [103]. A small study among UK women with the polycystic ovarian syndrome (PCOS) did not show a convincing association between serum and follicular fluid PFASs and sex steroids in PCOS patients [118]. A case-control study reported an association between exposure to PFAS and primary ovarian insufficiency among Chinese women. Also, exposure to PFOS and PFOA was associated with elevation of prolactin [117]. As summary, PFASs are probably associated with thyroid hormone leves in humans. Also, several studies showed association of PFASs and steroidogenesis and reproductive outcomes. However, more prospective studies with longitudinal design are necessary to clarify endocrine disrupting properties of PFASs.

13.6 Conclusions

Epidemiological studies examined the association of perinatal exposure to PFASs with prenatal growth mostly reported as inverse associations, although some studies did not reveal statistically significant associations. Some studies of postnatal growth suggested positive associations between PFAS exposure and postnatal weight gain or obesity. Several studies, but not all, found immunotoxic effects of PFASs in infants and children including reduced vaccine response, and increased risk of infectious diseases. However, the effects of PFASs on allergic conditions are not yet clear. Similarly, the associations between PFASs and neurodevelopmental outcomes are not conclusive, and the PFASs may only be weakly neurotoxic. Most of the studies have examined exposures to PFASs with 6–10 carbon length; only few studies have focused on PFASs with shorter and longer carbon chains. Animal studies suggest that PFASs with longer carbon chains may exhibit more toxicity and longer half-lives. Accordingly, results from

recent Asian studies with a high detection rate of long-chain PFASs show convincing associations with examined outcomes. Therefore, more studies are necessary to establish causal relationships between the full range of PFASs in human developmental exposures and their adverse health outcomes, preferably with a prospective design and larger sample size, examining a wide range of PFASs including long-chain PFASs and longer follow-up periods. Additionally, because of ubiquitous human exposure to POPs, examining multiple exposures and mixture models in future data analysis is warranted. Due to the lasting impact of adverse effects during early development, prevention of PFASs exposure should be regarded as a public health priority.

Acknowledgements This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18gk0110032.

References

1. ATSDR (2018). https://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237.
2. Pizzurro DM, Seeley M, Kerper LE, Beck BD. Interspecies differences in perfluoroalkyl substances (PFAS) toxicokinetics and application to health-based criteria. Regul Toxicol Pharmacol. 2019;106:239–50.
3. Grandjean P, Abdennebi-Najar L, Barouki R, Cranor CF, Etzel RA, Gee D, et al. Timescales of developmental toxicity impacting on research and needs for intervention. Basic Clin Pharmacol Toxicol. 2018;125(Suppl 3):70–80. https://doi.org/10.1111/bcpt.13162.
4. United States Environment Protection Agency (2019). https://www.epa.gov/pfas.
5. OECD (2002). http://www.oecd.org/chemicalsafety/risk-assessment/2382880.pdf.
6. OECD (2007). http://www.oecd.org/env/ehs/risk-management/perfluorooctanesulfonatepfosandrelatedchemicalproducts.htm.
7. Olsen GW, Burris JM, Ehresman DJ, Froehlich JW, Seacat AM, Butenhoff JL, et al. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ Health Perspect. 2007;115:1298–305. https://doi.org/10.1289/ehp.10009.
8. D'eon JC, Mabury SA. Is indirect exposure a significant contributor to the burden of perfluorinated acids observed in humans? Environ Sci Technol. 2011;45:7974–84.
9. Fromme H, Tittlemier SA, Völkel W, Wilhelm M, Twardella D. Perfluorinated compounds—exposure assessment for the general population in Western countries. Int J Hyg Environ Health. 2009;212:239–70.
10. Domingo JL. Health risks of dietary exposure to perfluorinated compounds. Environ Int. 2012;40:187–95.
11. Inoue K, Okada F, Ito R, Kato S, Sasaki S, Nakajima S, et al. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environ Health Perspect. 2004;112:1204–7. https://doi.org/10.1289/ehp.6864.
12. Midasch O, Drexler H, Hart N, Beckmann MW, Angerer J. Transplacental exposure of neonates to perfluorooctanesulfonate and perfluorooctanoate: a pilot study. Int Arch Occup Environ Health. 2007;80(7):643–8.

13. Apelberg BJ, et al. Cord serum concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in relation to weight and size at birth. Environ Health Perspect. 2007;115(11):1670–6.
14. Fei C, et al. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. Environ Health Perspect. 2007;115(11):1677–82.
15. Fei C, et al. Fetal growth indicators and perfluorinated chemicals: a study in the Danish National Birth Cohort. Am J Epidemiol. 2008;168(1):66–72.
16. Monroy R, et al. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environ Res. 2008;108(1):56–62.
17. Washino N, et al. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect. 2009;117(4):660–7.
18. Stein CR, Savitz DA, Dougan M. Serum levels of perfluorooctanoic acid and perfluorooctane sulfonate and pregnancy outcome. Am J Epidemiol. 2009;170(7):837–46.
19. Hamm MP, et al. Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol. 2010;20(7):589–97.
20. Whitworth KW, et al. Perfluorinated compounds in relation to birth weight in the Norwegian Mother and Child Cohort Study. Am J Epidemiol. 2012;175(12):1209–16.
21. Wu KS, et al. Association between maternal exposure to perfluorooctanoic acid (PFOA) from electronic waste recycling and neonatal health outcomes. Environ Int. 2012;48:1–8.
22. Chen MH, et al. Perfluorinated compounds in umbilical cord blood and adverse birth outcomes. PLoS One. 2012;7(8):e42474.
23. Maisonet M, et al. Maternal concentrations of polyfluoroalkyl compounds during pregnancy and fetal and postnatal growth in British girls. Environ Health Perspect. 2012;120(10):1432–7.
24. Kishi R, et al. The association of prenatal exposure to perfluorinated chemicals with maternal essential and long-chain polyunsaturated fatty acids during pregnancy and the birth weight of their offspring: the Hokkaido study. Environ Health Perspect. 2015;123(10):1038–45.
25. Bach CC, et al. Perfluoroalkyl acids in maternal serum and indices of fetal growth: the Aarhus birth cohort. Environ Health Perspect. 2016;124(6):848–54.
26. Lee ES, Han S, Oh JE. Association between perfluorinated compound concentrations in cord serum and birth weight using multiple regression models. Reprod Toxicol. 2016;59:53–9.
27. Starling AP, et al. 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. 2017;125(6):067016.
28. Sagiv SK, et al. Early pregnancy perfluoroalkyl substance plasma concentrations and birth outcomes in project viva: confounded by pregnancy hemodynamics? Am J Epidemiol. 2017;187(4):793–802.
29. Manzano-Salgado CB, et al. Prenatal exposure to perfluoroalkyl substances and birth outcomes in a Spanish birth cohort. Environ Int. 2017;108:278–84.
30. Li M, et al. Isomers of perfluorooctanesulfonate (PFOS) in cord serum and birth outcomes in China: Guangzhou Birth Cohort Study. Environ Int. 2017;102:1–8.
31. Chen MH, et al. The impact of prenatal perfluoroalkyl substances exposure on neonatal and child growth. Sci Total Environ. 2017;607-608:669–75.
32. Buck Louis GM, Zhai S, Smarr MM, Grewal J, Zhang C, Grants KL, et al. Endocrine disruptors and neonatal anthropometry, NICHD Fetal Growth Studies - Singletons. Environ Int. 2018;119:515–26.
33. Gyllenhammar I, Diderholm B, Gustafsson J, Berger U, Ridefelt P, Benskin JP, et al. Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environ Int. 2018;111:191–9.
34. Andersen CS, et al. Prenatal exposures to perfluorinated chemicals and anthropometric measures in infancy. Am J Epidemiol. 2010;172(11):1230–7.
35. Halldorsson TI, et al. Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study. Environ Health Perspect. 2012;120(5):668–73.

36. Andersen CS, et al. Prenatal exposures to perfluorinated chemicals and anthropometry at 7 years of age. Am J Epidemiol. 2013;178(6):921–7.
37. Braun JM, et al. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: the HOME study. Obesity (Silver Spring). 2016;24(1):231–7.
38. Mora AM, et al. Prenatal exposure to perfluoroalkyl substances and adiposity in early and mid-childhood. Environ Health Perspect. 2017;125(3):467–73.
39. Donauer S, et al. Prenatal exposure to polybrominated diphenyl ethers and polyfluoroalkyl chemicals and infant neurobehavior. J Pediatr. 2015;166(3):736–42.
40. Goudarzi H, et al. Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: the Hokkaido Study. Sci Total Environ. 2016;541:1002–10.
41. Chen MH, et al. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology. 2013;24(6):800–8.
42. Forns J, et al. Perfluoroalkyl substances measured in breast milk and child neuropsychological development in a Norwegian birth cohort study. Environ Int. 2015;83:176–82.
43. Gump BB, et al. Perfluorochemical (PFC) exposure in children: associations with impaired response inhibition. Environ Sci Technol. 2011;45(19):8151–9.
44. Stein CR, Savitz DA, Bellinger DC. Perfluorooctanoate and neuropsychological outcomes in children. Epidemiology. 2013;24(4):590–9.
45. Wang Y, et al. Prenatal exposure to perfluoroalkyl substances and children's IQ: the Taiwan maternal and infant cohort study. Int J Hyg Environ Health. 2015;218(7):639–44.
46. Vuong AM, et al. Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and executive function in school-age children. Environ Res. 2016;147:556–64.
47. Harris MH, Oken E, Rifas-Shiman SL, Calafat AM, Ye X, Bellinger DC, et al. Prenatal and childhood exposure to per-and polyfluoroalkyl substances (PFASs) and child cognition. Environ Int. 2018;115:358–69.
48. Liew Z, Ritz B, Bach CC, Asarnow RF, Bech BH, Nohr EA, et al. Prenatal exposure to perfluoroalkyl substances and IQ scores at age 5; a study in the Danish National Birth Cohort. Environ Health Perspect. 2018;126(6):067004.
49. Vuong AM, Yolton K, Wang Z, Xie C, Webster GM, Ye X, et al. Childhood perfluoroalkyl substance exposure and executive function in children at 8 years. Environ Int. 2018;119:212–9.
50. Zhang H, Yolton K, Webster GM, Ye X, Calafat AM, Dietrich KN, et al. Prenatal and childhood perfluoroalkyl substances exposures and children's reading skills at ages 5 and 8 years. Environ Int. 2018;2018(111):224–31.
51. Hoffman K, et al. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in U.S. children 12-15 years of age. Environ Health Perspect. 2010;118(12):1762–7.
52. Fei C, Olsen J. Prenatal exposure to perfluorinated chemicals and behavioral or coordination problems at age 7 years. Environ Health Perspect. 2011;119(4):573–8.
53. Stein CR, Savitz DA. Serum perfluorinated compound concentration and attention deficit/hyperactivity disorder in children 5-18 years of age. Environ Health Perspect. 2011;119(10):1466–71.
54. Braun JM, et al. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environ Health Perspect. 2014;122(5):513–20.
55. Ode A, et al. Fetal exposure to perfluorinated compounds and attention deficit hyperactivity disorder in childhood. PLoS One. 2014;9(4):e95891.
56. Stein CR, Savitz DA, Bellinger DC. Perfluorooctanoate exposure in a highly exposed community and parent and teacher reports of behaviour in 6-12-year-old children. Paediatr Perinat Epidemiol. 2014;28(2):146–56.
57. Strom M, et al. Persistent organic pollutants measured in maternal serum and offspring neurodevelopmental outcomesDOUBLEHYPHENa prospective study with long-term follow-up. Environ Int. 2014;68:41–8.

58. Hoyer BB, et al. Pregnancy serum concentrations of perfluorinated alkyl substances and offspring behaviour and motor development at age 5-9 years – a prospective study. Environ Health. 2015;14:2.
59. Liew Z, Ritz B, von Ehrenstein OS, Bech BH, Nohr EA, Fei C, et al. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case–control study in the Danish National Birth Cohort. Environ Health Perspect. 2015;123(4):367–73.
60. Lien GW, Huang CC, Shiu JS, Chen MH, Hsieh WS, Guo YL, Chen PC. Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere. 2016;156:118–27.
61. Oulhote Y, et al. Behavioral difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances. Environ Int. 2016;97:237–45.
62. Quaak I, et al. Prenatal exposure to perfluoroalkyl substances and behavioral development in children. Int J Environ Res Public Health. 2016;13(5):E511.
63. Lyall K, Yau VM, Hansen R, Kharrazi M, Yoshida CK, Calafat AM, et al. Prenatal maternal serum concentrations of per-and polyfluoroalkyl substances in association with autism spectrum disorder and intellectual disability. Environ Health Perspect. 2018;126(1):017001.
64. Fei C, et al. Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environ Health Perspect. 2008;116(10):1391–5.
65. Liew Z, et al. Prenatal exposure to perfluoroalkyl substances and the risk of congenital cerebral palsy in children. Am J Epidemiol. 2014;180(6):574–81.
66. Fei C, et al. Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. Environ Res. 2010;110(8):773–7.
67. Grandjean P, et al. Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA. 2012;307(4):391–7.
68. Grandjean P, Budtz-Jørgensen E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environ Health. 2013;12(1):35.
69. Granum B, et al. Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. J Immunotoxicol. 2013;10(4):373–9.
70. Mogensen UB, Grandjean P, Heilmann C, Nielsen F, Weihe P, Budtz-Jørgensen E. Structural equation modeling of immunotoxicity associated with exposure to perfluorinated alkylates. Environ Health. 2015;14:47.
71. Goudarzi H, et al. Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children. Environ Int. 2016;94:124–32.
72. Pennings JL, et al. Cord blood gene expression supports that prenatal exposure to perfluoroalkyl substances causes depressed immune functionality in early childhood. J Immunotoxicol. 2016;13(2):173–80.
73. Dalsager L, et al. Association between prenatal exposure to perfluorinated compounds and symptoms of infections at age 1-4years among 359 children in the Odense Child Cohort. Environ Int. 2016;96:58–64.
74. Grandjean P, et al. Serum vaccine antibody concentrations in adolescents exposed to perfluorinated compounds. Environ Health Perspect. 2017;125(7):077018.
75. Grandjean P, et al. Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. J Immunotoxicol. 2017;14(1):188–95.
76. Pilkerton CS, Hobbs GR, Lilly C, Knox SS. Rubella immunity and serum perfluoroalkyl substances: sex and analytic strategy. PLoS One. 2018;13(9):e0203330.
77. Wang IJ, et al. The effect of prenatal perfluorinated chemicals exposures on pediatric atopy. Environ Res. 2011;111(6):785–91.
78. Okada E, et al. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environ Res. 2012;112:118–25.

79. Dong GH, et al. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environ Health Perspect. 2013;121(4):507–13.
80. Humblet O, et al. Perfluoroalkyl chemicals and asthma among children 12-19 years of age: NHANES (1999-2008). Environ Health Perspect. 2014;122(10):1129–33.
81. Okada E, et al. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environ Int. 2014;65:127–34.
82. Osuna CE, et al. Autoantibodies associated with prenatal and childhood exposure to environmental chemicals in Faroese children. Toxicol Sci. 2014;142(1):158–66.
83. Ashley-Martin J, et al. Prenatal exposure to phthalates, bisphenol A and perfluoroalkyl substances and cord blood levels of IgE, TSLP and IL-33. Environ Res. 2015;140:360–8.
84. Smit LA, et al. Prenatal exposure to environmental chemical contaminants and asthma and eczema in school-age children. Allergy. 2015;70(6):653–60.
85. Buser MC, Scinicariello F. Perfluoroalkyl substances and food allergies in adolescents. Environ Int. 2016;88:74–9.
86. Stein CR, et al. Perfluoroalkyl and polyfluoroalkyl substances and indicators of immune function in children aged 12-19 y: National Health and Nutrition Examination Survey. Pediatr Res. 2016;79(2):348–57.
87. Zhu Y, Qin XD, Zeng XW, Paul G, Morawska L, Su MW, et al. Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: by gender and asthma status. Sci Total Environ. 2016;559:166–73.
88. Oulhote Y, et al. Children's white blood cell counts in relation to developmental exposures to methylmercury and persistent organic pollutants. Reprod Toxicol. 2017;68:207–14.
89. Qin XD, et al. Association of perfluoroalkyl substances exposure with impaired lung function in children. Environ Res. 2017;155:15–21.
90. Timmermann CA, et al. Association between perfluoroalkyl substance exposure and asthma and allergic disease in children as modified by MMR vaccination. J Immunotoxicol. 2017;14(1):39–49.
91. Zhou Y, et al. Perfluoroalkyl substance exposure and urine CC16 levels among asthmatics: a case-control study of children. Environ Res. 2017;159:158–63.
92. Zhou Y, Hu LW, Qian ZM, Geiger SD, Parrish KL, Dharmage SC, et al. Interaction effects of polyfluoroalkyl substances and sex steroid hormones on asthma among children. Sci Rep. 2017;7(1):899.
93. Chen Q, et al. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: a prospective birth cohort study. Environ Health. 2018;17(1):8.
94. Impinen A, Nygaard UC, Carlsen KL, Mowinckel P, Carlsen KH, Haug LS, Granum B. Prenatal exposure to perfluoroalkyl substances (PFASs) associated with respiratory tract infections but not allergy-and asthma-related health outcomes in childhood. Environ Res. 2018;160:518–23.
95. Goudarzi H, et al. Prenatal exposure to perfluoroalkyl acids and prevalence of infectious diseases up to 4years of age. Environ Int. 2017;104:132–8.
96. Zhu Y, et al. Associations of serum perfluoroalkyl acid levels with T-helper cell-specific cytokines in children: by gender and asthma status. Sci Total Environ. 2016;559:166–73.
97. Kim S, Choi K, Ji K, Seo J, Kho Y, Park J, et al. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environ Sci Technol. 2011;45(17):7465–72.
98. Webster GM, Venners SA, Mattman A, Martin JW. Associations between perfluoroalkyl acids (PFASs) and maternal thyroid hormones in early pregnancy: a population-based cohort study. Environ Res. 2014;133:338–47.
99. Wang Y, Rogan WJ, Chen PC, Lien GW, Chen HY, Tseng YC, et al. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ Health Perspect. 2014;122(5):529–34.

100. Berg V, Nøst TH, Hansen S, Elverland A, Veyhe AS, Jorde R, Odland JØ, Sandanger TM. Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environ Int. 2015;77:63–9.
101. Shah-Kulkarni S, Kim BM, Hong YC, Kim HS, Kwon EJ, Park H, Kim YJ, Ha EH. Prenatal exposure to perfluorinated compounds affects thyroid hormone levels in newborn girls. Environ Int. 2016;94:607–13.
102. Kato S, Itoh S, Yuasa M, Baba T, Miyashita C, Sasaki S, Nakajima S, Uno A, Nakazawa H, Iwasaki Y, Okada E, Kishi R. Association of perfluorinated chemical exposure in utero with maternal and infant thyroid hormone levels in the Sapporo cohort of Hokkaido Study on the Environment and Children's Health. Environ Health Prev Med. 2016;21(5):334–44.
103. Petersen MS, Halling J, Jørgensen N, Nielsen F, Grandjean P, Jensen TK, Weihe P. Reproductive function in a population of young Faroese men with elevated exposure to polychlorinated biphenyls (PCBs) and perfluorinated alkylate substances (PFAS). Int J Environ Res Public Health. 2018;15(9):E1880.
104. Dufour P, Pirard C, Seghaye MC, Charlier C. Association between organohalogenated pollutants in cord blood and thyroid function in newborns and mothers from Belgian population. Environ Pollut. 2018;238:389–96.
105. Aimuzi R, Luo K, Chen Q, Wang H, Feng L, Ouyang F, et al. Perfluoroalkyl and polyfluoroalkyl substances and fetal thyroid hormone levels in umbilical cord blood among newborns by prelabor caesarean delivery. Environ Int. 2019;130:104929.
106. Reardon AJF, Khodayari Moez E, Dinu I, Goruk S, Field CJ, Kinniburgh DW, MacDonald AM, Martin JW. APrON Study. Longitudinal analysis reveals early-pregnancy associations between perfluoroalkyl sulfonates and thyroid hormone status in a Canadian prospective birth cohort. Environ Int. 2019;129:389–99.
107. Itoh S, Araki A, Mitsui T, Miyashita C, Goudarzi H, Sasaki S, Cho K, Nakazawa H, Iwasaki Y, Shinohara N, Nonomura K, Kishi R. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. Environ Int. 2016;94:51–9.
108. Goudarzi H, Araki A, Itoh S, Sasaki S, Miyashita C, Mitsui T, Nakazawa H, Nonomura K, Kishi R. The association of prenatal exposure to perfluorinated chemicals with glucocorticoid and androgenic hormones in cord blood samples: the Hokkaido study. Environ Health Perspect. 2017;125(1):111–8.
109. Wang H, Du H, Yang J, Jiang H, Karmim O, Xu L, Liu S, Yi J, Qian X, Chen Y, Jiang Q, He G. PFOS, PFOA, estrogen homeostasis, and birth size in Chinese infants. Chemosphere. 2019;221:349–55.
110. Yao Q, Shi R, Wang C, Han W, Gao Y, Zhang Y, Zhou Y, Ding G, Tian Y. Cord blood per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood reproductive hormone. Environ Int. 2019;129:573–82.
111. Maisonet M, Calafat AM, Marcus M, Jaakkola JJ, Lashen H. Prenatal exposure to perfluoroalkyl acids and serum testosterone concentrations at 15 years of age in female ALSPAC study participants. Environ Health Perspect. 2015;123(12):1325–30.
112. Kristensen SL, Ramlau-Hansen CH, Ernst E, Olsen SF, Bonde JP, Vested A, Halldorsson TI, Becher G, Haug LS, Toft G. Long-term effects of prenatal exposure to perfluoroalkyl substances on female reproduction. Hum Reprod. 2013;28(12):3337–48.
113. Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE, Jørgensen N. Do perfluoroalkyl compounds impair human semen quality? Environ Health Perspect. 2009;117(6):923–7.
114. Joensen UN, Veyrand B, Antignac JP, Blomberg Jensen M, Petersen JH, et al. PFOS (perfluorooctanesulfonate) in serum is negatively associated with testosterone levels, but not with semen quality, in healthy men. Hum Reprod. 2013;28(3):599–608.
115. Barrett ES, Chen C, Thurston SW, Haug LS, Sabaredzovic A, Fjeldheim FN, et al. Perfluoroalkyl substances and ovarian hormone concentrations in naturally cycling women. Fertil Steril. 2015;103(5):1261–70.e3.

116. Vested A, Ramlau-Hansen CH, Olsen SF, Bonde JP, Kristensen SL, Halldorsson TI, et al. Associations of in utero exposure to perfluorinated alkyl acids with human semen quality and reproductive hormones in adult men. Environ Health Perspect. 2013;121(4):453–8.
117. Zhang S, Tan R, Pan R, Xiong J, Tian Y, Wu J, Chen L. Association of perfluoroalkyl and polyfluoroalkyl substances with premature ovarian insufficiency in Chinese women. J Clin Endocrinol Metab. 2018;103(7):2543–51.
118. Heffernan AL, Cunningham TK, Drage DS, Aylward LL, Thompson K, Vijayasarathy S, et al. Perfluorinated alkyl acids in the serum and follicular fluid of UK women with and without polycystic ovarian syndrome undergoing fertility treatment and associations with hormonal and metabolic parameters. Int J Hyg Environ Health. 2018;221(7):1068–75.
119. Preston EV, Webster TF, Oken E, Claus Henn B, McClean MD, Rifas-Shiman SL, et al. Maternal plasma per- and polyfluoroalkyl substance concentrations in early pregnancy and maternal and neonatal thyroid function in a prospective birth cohort: project viva (USA). Environ Health Perspect. 2018;126(2):027013.
120. Kim MJ, Moon S, Oh BC, Jung D, Ji K, Choi K, Park YJ. Association between perfluoroalkyl substances exposure and thyroid function in adults: a meta-analysis. PLoS One. 2018;13(5):e0197244.

Chapter 14
Brominated Flame Retardants (BFRs)

Kyungho Choi and Sunmi Kim

Abstract Evidence for adverse effects of brominated flame retardants (BFRs) in humans is reviewed, with a focus on polybrominated diphenyl ethers (PBDEs). BFRs may easily leach out during manufacture or the use of consumer products, and enter the environment. Food consumption or ingestion of contaminated dust is among the major pathways of human exposure to BFRs. Epidemiological and experimental reports suggest that exposure to BFRs may induce adverse neurodevelopmental, metabolic, and reproductive effects.

The sex hormone-related effects of BRFs include those related to birth outcomes, growth, and reproductive system. Moreover, several other health consequences such as neurodevelopmental and behavioral disorders, thyroid hormone system, and obesity were identified as endocrine effects of BRFs. Some studies reported conflicting observations; however, their thyroid hormone disruption and neurodevelopmental toxicities have been demonstrated frequently. The use of certain BFRs is banned worldwide, however, BFRs are persistent in the ecosystem and are accumulating in human because of their lipophilicity. Thus, active epidemiological and mechanistic studies, especially on the susceptible populations, are warranted.

Keywords Flame retardants · PBDEs · Birth outcomes · Congenital malformation Neurodevelopmental toxicity · Reproductive toxicity · Thyroid hormone disruption Obesity · Diabetes

14.1 Background

Brominated flame retardants (BFRs) are a group of brominated chemicals that have been used as flame retardant. When heated, BFRs release free bromine atoms and respond to free radicals generated during burning, and thus delay the combustion

K. Choi (✉) · S. Kim
Seoul National University, School of Public Health, Seoul, South Korea
e-mail: kyungho@snu.ac.kr

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_14

process. Because of this property, this group of compounds have been widely used in various consumer products such as electronics, furnishing, textiles, building materials, and polyurethane foams, to enhance ignition resistance. The use of BFRs has led to frequent detection of several major BRFs, such as tetrabromobisphenol A (TBBPA), polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), and hexabromocyclododecanes (HBCDs), in the environmental matrices and humans [1].

PBDEs are an important group of BFRs which have been used in a huge amount worldwide. The total production of PBDE commercial mixtures from 1970 to 2005 is estimated between 1.29 and 1.47 million tonnes worldwide [2]. PBDEs can be classified by the degree of bromination, and depending on the degree and location of bromination, a total of 209 congeners are possible. Among them, the most commonly used commercial PBDE mixtures are in forms of penta-, octa-, and deca-BDE. The most abundant PBDE congeners detected in biota and environment are BDE-47, -99, -100, -153, and -209 (Fig. 14.1).

Because of their structural similarity to T4, it is hypothesized that PBDEs might interfere with thyroid hormone transport and metabolism [3, 4]. Animal experimental studies support the thyroid disrupting effects of PBDEs [5]. As a possible consequence of thyroid disruption, their neurodevelopmental toxicity has been also suggested in both experimental organisms and humans [6]. Owing to the health concerns and their persistent nature, some PBDEs such as hexabromodiphenyl ether and heptabromodiphenyl ether (hexa- and hepta-BDE), tetrabromodiphenyl ether, penta-bromodiphenyl ether (tetra- and penta-BDE), and most recently, deca-BDE were listed in Annex A of the Stockholm Convention as persistent organic pollutants (POPs). Therefore, global efforts have been implemented to eliminate the production and the use of PBDEs. However, given the widespread use of this group of compounds and their persistent nature, PBDEs in the environment and biota are expected to be a lingering public health threat for decades to come.

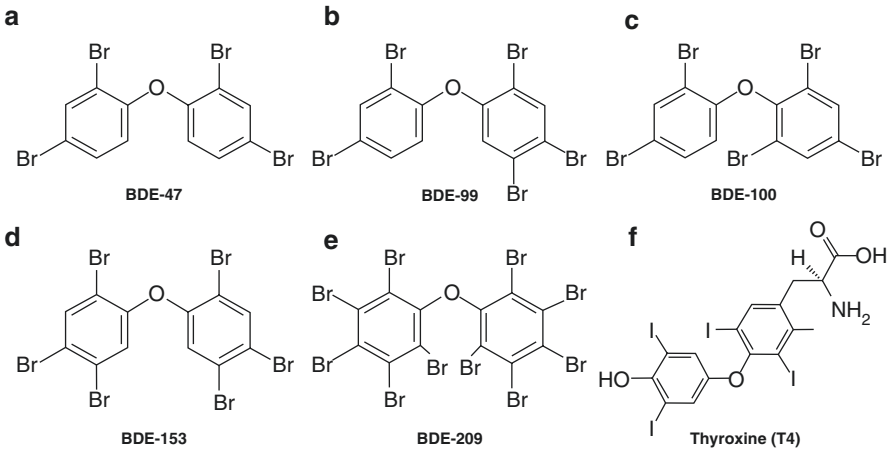


Fig. 14.1 Chemical structures of some major PBDE congeners and thyroxine

Unlike reactive flame retardants (FRs) such as TBBPA, which are covalently bonded into the polymer matrix, PBDEs are additive FRs, which are mixed with the polymer and do not form chemical bonds with the materials [1]. Both additive and reactive FRs can be easily leached out during manufacture or the use of consumer products and subsequently enter the environment. Humans are exposed to PBDEs through ingestion or inhalation of contaminated media. Intake of PBDEs is believed to occur primarily from food consumption, and to a lesser extent through air inhalation and dermal absorption [7–10]. Owing to their bio-accumulative characteristics, PBDEs are expected to be high in seafood, and hence humans with high seafood consumption tend to be exposed more [11]. Dust is another important route of oral exposure [8, 12–15]. In a recent Korean study, it was reported that incidental dust ingestion was major in children while food was more important source of exposure in adults [16].

The developing fetus may be exposed to PBDEs through the placenta [17]. For breastfed infants, breast milk is the most critical source of PBDE exposure [17, 18]. Because many plastic-made toys contain PBDEs, toys may be an important source of exposure to PBDEs in toddlers and young children [19, 20]. Specific behavior patterns of young children, like sucking and crawling, can increase the amount of exposure to PBDEs from the toys [19, 21]. Consequently, several PBDEs were detected at higher concentrations in the serum of toddlers compared to those of adults [22].

14.2 Human Toxicity of Brominated Chemicals

Knowledge about the human toxicity of PBDEs has accumulated in the last two decades. The extent of bromination appears to determine the toxicity of PBDEs, with more brominated congeners being less toxic. Thus, PBDEs found in the environment are likely to be more toxic than the forms which were applied to the products, because debromination occurs when they are released into the environment. Epidemiological evidence suggests an association between endocrine disruption and the developmental effects of PBDEs, even though, often, the observations are not consistent and the underlying etiology is not fully understood. Of particular concern is their association with adverse outcomes in neurobehavioral development among children.

This chapter describes what has been documented as human toxicity for PBDEs in detail, with a focus on endocrine disruption effects. Underlying toxicity mechanisms suggested for these outcomes are also briefly described.

14.2.1 Birth Outcomes

Several epidemiological studies reported the associations between prenatal exposure to PBDEs and birth outcomes [23–32]. Most studies used PBDE levels detected in maternal or cord blood serum to indicate prenatal exposure, and the levels measured in breast milk to represent postnatal exposure.

The associations between PBDE exposure and birth outcomes have been frequently reported worldwide. In many populations, prenatal exposure to PBDEs has been associated with adverse birth outcomes. In a Spanish cohort study, the sum of PBDE concentrations in cord serum was negatively associated with abdominal circumference and the fetal weight estimated at gestational week 12 [23]. In addition, negative associations of maternal serum PBDE concentrations in the first trimester with head circumference and birth weight were reported in the same cohort [23]. Similar observations were reported in China, e.g., negative associations between maternal BDE-28/-100 concentrations and birth length, and BDE-28 concentrations and birth weight [24]. More recently, cord blood concentrations of PBDEs have been shown to be associated with increased head circumference in Chinese prospective birth cohort [25]. In the US general population (N = 234), the association between maternal and paternal PBDE concentrations measured before conception and birth size has been reported. Interestingly, a significant association of both maternal and paternal PBDEs with lower birth weight was found among female infants, whereas paternal concentrations of PBDEs were associated with higher birth weight in boys [26]. In a cohort of pregnant mothers in the USA (N = 286), negative associations were observed between major PBDEs including BDE-47, 99, and 100 and birth weight, even though the significance disappeared when the maternal weight gain was added to the association model [27]. However, in the same study, prenatal exposure to PBDE did not show an association with birth length, head circumference, or gestational duration [27].

Lactational exposure to PBDEs was associated with reduced birth weight, birth length, and chest circumference in a Taiwanese women population [28]. In the USA, weak associations between early-life PBDE exposures via breast milk and anthropometric measurements were shown. However, in this population, weight-to-height z-scores were inversely associated with PBDEs in breast milk among boys; on the contrary, weight-for-height z-scores were positively associated with PBDEs, except for BDE-153, among girls [29]. In a Swedish cohort (1996–2010), maternal breast milk concentrations of PBDEs (sum of BDE-47, 99, 100 and 153) showed an inverse association with birth weight, and the associations became stronger among boys [30]. In Northern Tanzania, BDE-47, -99, -100, and -153 concentrations measured in colostrum were significantly correlated with birth weight and birth length [31].

The associations with preterm birth or gestational age are seldom investigated. One report suggests that high levels of maternal BDE-47 might increase the risk of preterm birth [32]. On the contrary, cord blood concentrations of PBDEs were associated with increased gestational age in Chinese birth cohort, i.e., 0.73 weeks increase for 1 log unit increase of PBDEs [25]. It is not clear whether PBDEs are associated with the gestational period, because only a small number of studies have been conducted, and the directions of the association are conflicting.

Despite accumulating evidence from epidemiological and cross-sectional studies, mechanisms underlying the association between PBDEs and birth outcome are not well understood. For example, maternal BMI or weight gain during pregnancy has been associated with fetal growth, and these maternal somatic characteristics can be influenced by various factors other than PBDEs. Further research is needed to study

whether PBDEs affect fetal growth independently, apart from maternal BMI and weight gain during pregnancy. Moreover, biological mechanisms underlying different responses by the infant sex are not known, and warrant further experimental studies.

14.2.2 Cryptorchidism and Hypospadias

Incidences of congenital malformation among male infants, including cryptorchidism and hypospadias, have been increasing significantly. It is suspected that cryptorchidism and hypospadias may share common risk factors [33]. Chemical-induced sex hormone disruption is one reason for these malformations, as it depends on the fetal conversion of testosterone to dihydrotestosterone, binding of dihydrotestosterone to the androgen receptor, and proper subsequent androgen receptor signaling [34]. The epidemiological studies have suggested associations of flame retardant exposure with genital malformation. Breast milk PBDE concentrations were found to be significantly higher in the boys with cryptorchidism than in controls, in a prospective Danish-Finnish study, 1997–2001 [35]. In a case–control study of Canada, concentrations of BDE-99, 100, and 154 in maternal hair were found to be significantly higher in the cases [36]. In another Canadian population, in utero exposure to PBDEs, as measured in maternal hair, was found to be higher by 48% in mothers who gave birth to infants with hypospadias [37]. Exposure to PBDEs that were measured in cord plasma samples also has been shown to be inversely associated with anogenital distance at birth, 6 months, 12 months, and 48 months of age in Shanghai birth cohort [38]. Considering the anti-androgenic potentials of PBDEs, not only congenital malformation but also adverse consequences in later life stages, warrant further investigations.

14.2.3 Neurodevelopment and Neurobehavioral Disorders

Prenatal exposure to PBDEs has been associated with alterations in behavioral domains, especially motor activity and cognitive function in later stages of life. This association was first reported in a US based study, which showed that children with higher cord serum concentrations of PBDEs scored lower mental and physical test scores at 12–48 and 72 months of age [39]. Subsequently, a number of epidemiological studies of similar design have been published. Maternal serum BDE-47 levels were associated with internalizing and externalizing problems in the Child Behavior Checklist, in toddlers of 18–24 months of age, in Korea [40]. In a Chinese population, cord serum BDE-99 and BDE-47 concentrations were observed to be significantly associated with lower language developmental index and social developmental index, respectively, at 24 months of age, but not at 12 months [41]. In a US population, prenatal exposure to BDE-47 was also shown to be associated with attention problems at 3–7 years of age [42].

Postnatal exposure also has been found to be associated with neurodevelopmental indices in several studies. In a Taiwanese infant population ($N = 70$), elevated PBDE levels in breast milk were shown to be associated with developmental delays in cognition [43]. In a North Carolina study cohort ($N = 222$), lactational PBDE exposure was observed to be correlated with increased activity and impulsive behavior in early childhood [29]. However, there are conflicting observations as well. In a prospective birth cohort in Spain ($N = 88$ for cord blood samples, and $N = 244$ for serum at age 4), no association was seen between PBDE body-burden and motor or cognitive alteration in 4-year-old children [44]. In Belgium adolescents ($N = 515$), no significant association was observed between serum PBDEs, HBCD, and TBBPA concentrations and attention, visual scanning, or working memory test scores. The only exception is the motor function scores in the finger tapping test which were shown to only be significantly associated with serum PBDEs levels in this population, showing a decrease in the number of taps by 5.31, by a two-fold increase of the sum of serum PBDEs [45].

In a California birth cohort, which was conducted in highly contaminated regions of the USA, both prenatal and childhood PBDE exposures were shown to be associated with poor attention, fine motor coordination, and cognition (Full-Scale IQ) at 7 years of age [46]. Several other studies have reported significant associations of prenatal and postnatal PBDE exposure with neurodevelopmental indices in the Health Outcomes and Measures of the Environment (HOME) Study. In the HOME study, maternal serum BDE-28 concentrations were associated with autistic behavior at 4–5 years of age [47], and BDE-47 concentrations were observed to be associated with a decrease in intelligence quotient (IQ) at 5 years of age [48]. However, neither psychomotor nor mental indices of the Bayley Scales of Infant Development-II (BSID-II) at ages 1, 2, and 3 years were shown to be significantly associated with prenatal PBDEs exposure in this cohort [48]. Recently, a follow-up of the HOME study showed that PBDEs concentrations measured in children of 8 years old were significantly associated with poorer emotional and impulse control [49], but those measured in children of 1, 2, 3, and 5 years old did not show an association. The results of the HOME study suggest that at the environmentally relevant levels of exposure, potential of neurodevelopmental toxicity cannot be ignored among children.

The exact mechanisms underlying PBDE neurotoxicity are unclear, but generally, two modes of action affecting brain development have been suggested [6]. One mode of action is a capacity of PBDEs to alter thyroid hormone homeostasis which can eventually result in brain development. The other possibilities include the oxidative stress potential of PBDEs, disruption of calcium signal transduction, and decrease in neural and oligodendrocyte differentiation, thereby affecting nervous system cells directly.

14.2.4 Reproductive Systems

Toxicities of PBDEs on the human reproductive system have not thoroughly studied, and, therefore, there remain gaps in knowledge. Among various reproductive dysfunction indicators, menstruation characteristics and age at puberty have been

studied among female population. In Taiwan, PBDE concentrations in breast milk were not associated with maternal menstruation characteristics (N = 20), even though their concentrations were shown to be significantly related to the birth size of infants [28]. In another study conducted in Taiwanese women (N = 46), higher concentrations of PBDEs were shown to be significantly associated with prolonged length of menstrual cycle and irregular menstruation periods [50]. Menstruation characteristics are closely related to fertility. Age at puberty is also an important indicator of reproductive system toxicity in humans. One longitudinal study in the USA explored the association between pubertal timing and PBDE exposure. In this study, the age at pubertal transition was observed to be significantly higher among girls with greater PBDE levels [51]. Among a small male population recruited through a US infertility clinic (N = 62), positive associations of house dust penta- and octa-PBDE concentrations with hormone levels of estradiol, and sex hormone binding globulin (SHBG), luteinizing hormone (LH) and testosterone were observed, although an inverse association of deca-BDE concentrations with testosterone was seen [52].

14.2.5 Thyroid Hormones

Thyroid hormones play a crucial role in the maintenance and activation of metabolic function, neurodevelopment, and cognitive function. Moderate changes in thyroid hormone levels during pregnancy may be associated with adverse outcomes in offspring [53, 54]. For example, significantly lower IQ scores were found in children of women with thyroid deficiency during pregnancy, even though hormone levels were found within the reference range [55].

Significant associations between PBDEs exposure and thyroid hormone levels among adults are summarized in Table 14.1. Thyroid hormone disruptive effect of PBDEs has been suggested in diverse populations including the general population,

Table 14.1 Summary of associations between PBDE concentrations in serum and thyroid hormone levels reported in adults or pregnant women

Target population	N	Thyroid hormone					Reference [No.]
		fT3	TT3	fT4	TT4	TSH	
Adults	405			↑	↑	↓	Turyk et al. (2008) [56]
Adults	623		↑				Dallaire et al. (2009) [57]
Adults	325	↓	↓	↓		↓	Wang et al. (2010) [58]
Adult, women	745				↓	↑	Oulhote et al. (2016) [59]
Pregnant women	270			–		↓	Chevrier et al. (2010) [60]
Pregnant women	140	–	↑	↑	↑	–	Stapleton et al. (2011) [61]
Pregnant women	380	↓	↓	↓	↓	–	Abdelouahab et al. (2013) [62]
Pregnant women	105	–	–	↓	↓	↑	Kim et al. (2013) [63]
Pregnant women	187	↑	↑	↑	↑		Vuong et al. (2015) [64]

'–' no association; '↑' positive association; '↓' negative association; blank indicates data not provided or available. 'fT3' free T3; 'TT3' total T3; 'fT4' free T4; 'TT4' total T4. Studies with target population of $N > 100$ are included in the table

fish consumers, and workers [56–58]. In recent studies, hypothyroidism, i.e., low T4 and high TSH, was shown to be associated with higher PBDE concentrations [59]. One study reported significant associations between PBDE concentrations and low status of free T3, total T3, free T4, and TSH simultaneously, but the participants were recruited from highly contaminated and occupational exposure conditions (e-waste site) [60]. Similarly, one large population study based on Inuit adults showed that exposure to BDE-47 was positively associated with total T3 [61].

Among pregnant women, the associations between PBDEs and subclinical hypothyroidism or hyperthyroidism were reported in several studies. For example, higher PBDE exposure were associated with lower TSH [62] or higher thyroid hormone levels without lower TSH [63, 64] in pregnant women. Significant disruption of thyroid hormone homeostasis by PBDEs exposure was shown in other studies based on pregnant women as well [65, 66].

The adverse effects of prenatal expsoure in newborn infants have been documented. Herbstman et al. [67] found significant association between cord serum PBDEs and lower T4 or higher TSH among babies born by spontaneous vaginal delivery. Although evidence showing otherwise is often found [68], most studies with neonatal population show significant associations between PBDEs exposure and hypothyroidism, e.g., a decrease in thyroid hormones or increased TSH (Table 14.2). Prenatal PBDE exposure was inversely associated with cord blood free T4 and total T4 in a large population-based study ($N = 260$) [65]. In addition, PBDEs exposure was inversely associated with free T3 and total T3 in cord blood serum in a small population-based study ($N = 50$) [69]. In both studies, however TSH was not influenced by PBDE level. In Korean general population, however, PBDE exposure as measured in cord blood serum was associated with increased TSH in newborn infants without change in T3 and T4 [70].

The adverse effects of PBDEs on thyroid function have also been reported in children and post-menopausal women. In children of China, serum PBDE concentrations were associated with increased T3 [71]. Unlike previous reports, the direc-

Table 14.2 Summary of associations between prenatal PBDE concentrations and thyroid hormone levels of newborn infants or children

Target population	Matrix for PBDEs measurement	*N*	Thyroid hormone					Reference [No.]
			fT3	TT3	fT4	TT4	TSH	
Newborn infants	Cord serum	297			–	↓	↑	Herbstman et al. (2008) [67]
Newborn infants	Maternal serum	260	–	–	↓	↓	–	Abdelouahab et al. (2013) [62]
Newborn infants	Cord serum	104	–	–	–	–	↑	Kim et al. (2015) [69]
Newborn infants	Maternal Serum	104	–	–	–	–	↑	
Children	Serum	T74	↑	–	–	–	–	Guo et al. (2018) [70]

'–' no association; '↑' positive association; '↓' negative association; blank indicates data not provided or available. 'fT3' free T3; 'TT3' total T3; 'fT4' free T4; 'TT4' total T4. Studies with target population of $N > 100$ are included in the table. Those reported null associations were not shown [68]

tion of thyroid hormone change is toward hyperthyroidism, and hence this observation warrants further validations in other children populations. Because thyroid hormones are in apparent crosstalk with estrogens [72], menopausal status of women may influence thyroid hormone disruption by exogenous chemicals, due to the difference in estrogen reserves. One study based on the NHANES data suggested that general adult women with higher PBDE concentrations exhibited increased odds of having thyroid disease. In stratified analysis, this association became stronger in post-menopausal population, suggesting enhanced effects of PBDEs on thyroid signaling by the lowered estrogen levels [73]. In contrast, but partly supporting the crosstalk between thyroid and sex hormones, premenopausal adult women aged between 30 and 50 years of age showed stronger associations between PBDE exposure levels and prevalence of hypothyroidism, compared to older women aged between 51 and 79 years old [59].

PBDEs share structural similarity with T4 (Fig. 14.1) and cause thyroid hormone disruption through alteration of transport and metabolism/deactivation of thyroid hormones. UDP-GT induction catalyzes the glucuronidation of T4, eventually leading to the clearance of circulating T4 [74, 75]. Circulating T4 hormone may competitively binds to the thyroid hormone transport protein [76] and thyroid hormone receptor [77], and therefore has a potential to interfere with the normal transport of thyroid hormones to peripheral tissues, and inhibits cellular uptake of thyroid hormones in thyroid hormone-sensitive cells.

14.2.6 Obesity and Diabetes

Obesity, diabetes and other metabolic dysfunctions are closely related to the thyroid hormone system. Obesity and related diseases are therefore associated with chemicals disrupting thyroid function, e.g., BFRs including PBDEs. The associations of PBDEs and PBBs with diabetes and metabolic syndrome have been reported in a population participating in the US NHANES 2003–2004 [78]. In mothers, who participated in the Salinas birth cohort ($N = 468$), positive associations with BMI were observed for BDE-47, while BDE-153 was shown to be inversely associated [79]. Most recently, a very large French cohort ($N = 71{,}415$) showed that HBCD and PBDEs exposure were associated with type 2 diabetes (T2D) risk [80]. Among children of a California birth cohort ($N = 224$), prenatal exposure to total PBDEs showed positive associations with BMI z-score in boys, while a negative association in girls at age 7 years was observed [81]. However, children's serum BDE-153 concentrations were inversely associated with BMI [81]. Similar negative associations were also observed in the HOME study. PBDEs during pregnancy were shown to be associated with anthropometric measures in children aged 1–8 years ($N = 318$), while maternal serum PBDEs were shown to be associated with lower BMI z-score, decreased waist circumference, and lower percent body fat [82]. In HOME study participants, at age 8 years, ($N = 206$), BDE-153 concentrations in children's serum were shown to be inversely

associated with adiposity measures, but no significant association was found for BDE-28, -47, -99, and -100 [83]. In both studies, negative associations between BDE-153 concentrations and adiposity measures were clear [82, 83]. For BDE-153, the mechanisms involved in reduction of adipose tissue are unknown. For example, a significant increase in serum BDE-153 levels has been shown after weight loss treatments in a group of 94 obese adolescents, without changes in BDE-47 and -100 [84]. BDE-153 has a longer half-life than BDE-47, -99, and -100 [85], leading to greater storage of PBDEs in the adipose tissue of person with higher adiposity. Thus, while experimental studies suggest obesogenic effects of PBDEs [86, 87], reverse causality may be observed in cross-sectional studies.

14.3 Conclusions

Epidemiological evidence indicating endocrine disruption by PBDEs are accumulating, even though inconsistent observations are also reported. PBDEs are associated with thyroid hormone disruption and negative neurodevelopmental outcomes in many populations including newborn children and pregnant women. However, their associations with sex hormone disruption or obesity are less studied and uncertain. Studies on populations at high levels of exposure such as e-waste disposal site residents or people with occupational BFR exposure are also warranted.

References

1. de Wit CA, Herzke D, Vorkamp K. Brominated flame retardants in the Arctic environment-trends and new candidates. Sci Total Environ. 2010;408(15):2885–918.
2. UNEP (2010) Supporting document for technical review of the implications of recycling commercial pentabromodiphenyl ether and commercial octabromodiphenyl ether. Stockholm Convention document for 6th POP Reviewing Committee meeting (UNEP/POPS/POPRC.6/INF/6). Available at http://chm.pops.int/Default.aspx?tabid=783 [Accessed 31 March 2019].
3. Birnbaum LS, Staskal DF. Brominated flame retardants: cause for concern? Environ Health Perspect. 2004;112(1):9–17.
4. McDonald TA. A perspective on the potential health risks of PBDEs. Chemosphere. 2002;46(5):745–55.
5. Boas M, Feldt-Rasmussen U, Main KM. Thyroid effects of endocrine disrupting chemicals. Mol Cell Endocrinol. 2012;355(2):240–8.
6. Costa LG, de Laat R, Tagliaferri S, Pellacani C. A mechanistic view of polybrominated diphenyl ether (PBDE) developmental neurotoxicity. Toxicol Lett. 2014;230(2):282–94.
7. Allen JG, McClean MD, Stapleton HM, Nelson JW, Webster TF. Personal exposure to polybrominated diphenyl ethers (PBDEs) in residential indoor air. Environ Sci Technol. 2007;41(13):4574–9.
8. Harrad S, Hazrati S, Ibarra C. Concentrations of polychlorinated biphenyls in indoor air and polybrominated diphenyl ethers in indoor air and dust in Birmingham, United Kingdom: implications for human exposure. Environ Sci Technol. 2006;40(15):4633–8.

9. Wu N, Herrmann T, Paepke O, Tickner J, Hale R, Harvey LE, La Guardia M, McClean MD, Webster TF. Human exposure to PBDEs: associations of PBDE body burdens with food consumption and house dust concentrations. Environ Sci Technol. 2007;41(5):1584–9.
10. Bramwell L, Glinianaia SV, Rankin J, Rose M, Fernandes A, Harrad S, Pless-Mulolli T. Associations between human exposure to polybrominated diphenyl ether flame retardants via diet and indoor dust, and internal dose: a systematic review. Environ Int. 2016;92-93:680–94.
11. Schecter A, Papke O, Tung KC, Staskal D, Birnbaum L. Polybrominated diphenyl ethers contamination of United States food. Environ Sci Technol. 2004;38(20):5306–11.
12. Jones-Otazo HA, Clarke JP, Diamond ML, Archbold JA, Ferguson G, Harner T, Richardson GM, Ryan JJ, Wilford B. Is house dust the missing exposure pathway for PBDEs? An analysis of the urban fate and human exposure to PBDEs. Environ Sci Technol. 2005;39(14):5121–30.
13. Zota AR, Rudel RA, Morello-Frosch RA, Brody JG. Elevated house dust and serum concentrations of PBDEs in California: unintended consequences of furniture flammability standards? Environ Sci Technol. 2008;42(21):8158–64.
14. Stapleton HM, Eagle S, Sjodin A, Webster TF. Serum PBDEs in a North Carolina toddler cohort: associations with handwipes, house dust, and socioeconomic variables. Environ Health Perspect. 2012;120(7):1049–54.
15. Lorber M. Exposure of Americans to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol. 2008;18(1):2–19.
16. Lee S, Kannan K, Moon HB. Assessment of exposure to polybrominated diphenyl ethers (PBDEs) via seafood consumption and dust ingestion in Korea. Sci Total Environ. 2013;443:24–30.
17. Shin MY, Kim S, Lee S, Kim HJ, Lee JJ, Choi G, Choi S, Kim S, Kim SY, Park J, Moon HB, Choi K, Kim S. Prenatal contribution of 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47) to total body burden in young children. Sci Total Environ. 2018;616-617:510–6.
18. Lee S, Kim S, Kim E, Lee IS, Choi G, Kim HJ, Park J, Jae Lee J, Choi S, Young Kim S, Kim S, Kim S, Choi K, Moon HB. Polybrominated diphenyl ethers (PBDEs) in breast milk of Korea in 2011: current contamination, time course variation, influencing factors and health risks. Environ Res. 2013;126:76–83.
19. Chen SJ, Ma YJ, Wang J, Chen D, Luo XJ, Mai BX. Brominated flame retardants in children's toys: concentration, composition, and children's exposure and risk assessment. Environ Sci Technol. 2009;43(11):4200–6.
20. Ionas AC, Dirtu AC, Anthonissen T, Neels H, Covaci A. Downsides of the recycling process: harmful organic chemicals in children's toys. Environ Int. 2014;65:54–62.
21. Hoffman K, Webster TF, Sjodin A, Stapleton HM. Toddler's behavior and its impacts on exposure to polybrominated diphenyl ethers. J Expo Sci Environ Epidemiol. 2017;27(2):193–7.
22. Toms LM, Sjodin A, Harden F, Hobson P, Jones R, Edenfield E, Mueller JF. Serum polybrominated diphenyl ether (PBDE) levels are higher in children (2–5 years of age) than in infants and adults. Environ Health Perspect. 2009;117(9):1461–5.
23. Lopez-Espinosa MJ, Costa O, Vizcaino E, Murcia M, Fernandez-Somoano A, Iniguez C, Llop S, Grimalt JO, Ballester F, Tardon A. Prenatal exposure to polybrominated flame retardants and fetal growth in the INMA cohort (Spain). Environ Sci Technol. 2015;49(16):10108–16.
24. Chen L, Wang C, Cui C, Ding G, Zhou Y, Jin J, Gao Y, Tian Y. Prenatal exposure to polybrominated diphenyl ethers and birth outcomes. Environ Pollut. 2015;206:32–7.
25. Chen L, Wang C, Zhang Y, Zhou Y, Shi R, Cui C, Gao Y, Tian Y. Polybrominated diphenyl ethers in cord blood and perinatal outcomes from Laizhou Wan Birth Cohort, China. Environ Sci Pollut Res Int. 2018;25(21):20802–8.
26. Robledo CA, Yeung E, Mendola P, Sundaram R, Maisog J, Sweeney AM, Barr DB, Louis GM. Preconception maternal and paternal exposure to persistent organic pollutants and birth size: the LIFE study. Environ Health Perspect. 2015;123(1):88–94.
27. Harley KG, Chevrier J, Aguilar Schall R, Sjodin A, Bradman A, Eskenazi B. Association of prenatal exposure to polybrominated diphenyl ethers and infant birth weight. Am J Epidemiol. 2011;174(8):885–92.

28. Chao HR, Wang SL, Lee WJ, Wang YF, Papke O. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from Central Taiwan and their relation to infant birth outcome and maternal menstruation effects. Environ Int. 2007;33(2):239–45.
29. Hoffman K, Adgent M, Goldman BD, Sjodin A, Daniels JL. Lactational exposure to polybrominated diphenyl ethers and its relation to social and emotional development among toddlers. Environ Health Perspect. 2012;120(10):1438–42.
30. Lignell S, Aune M, Darnerud PO, Hanberg A, Larsson SC, Glynn A. Prenatal exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) may influence birth weight among infants in a Swedish cohort with background exposure: a cross-sectional study. Environ Health. 2013;12:44.
31. Muller MH, Polder A, Brynildsrud OB, Lie E, Loken KB, Manyilizu WB, Mdegela RH, Mokiti F, Murtadha M, Nonga HE, Skaare JU, Lyche JL. Brominated flame retardants (BFRs) in breast milk and associated health risks to nursing infants in northern Tanzania. Environ Int. 2016;89-90:38–47.
32. Peltier MR, Koo HC, Getahun D, Menon R. Does exposure to flame retardants increase the risk for preterm birth? J Reprod Immunol. 2015;107:20–5.
33. Akre O, Lipworth L, Cnattingius S, Sparen P, Ekbom A. Risk factor patterns for cryptorchidism and hypospadias. Epidemiology. 1999;10(4):364–9.
34. Carmichael SL, Herring AH, Sjodin A, Jones R, Needham L, Ma C, Ding K, Shaw GM. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere. 2010;80(6):641–6.
35. Main KM, Kiviranta H, Virtanen HE, Sundqvist E, Tuomisto JT, Tuomisto J, Vartiainen T, Skakkebaek NE, Toppari J. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environ Health Perspect. 2007;115(10):1519–26.
36. Goodyer CG, Poon S, Aleksa K, Hou L, Atehortua V, Carnevale A, Koren G, Jednak R, Emil S, Bagli D, Dave S, Hales BF, Chevrier J. A case-control study of maternal polybrominated diphenyl ether (PBDE) exposure and cryptorchidism in Canadian populations. Environ Health Perspect. 2017;125(5):057004.
37. Poon S, Koren G, Carnevale A, Aleksa K, Ling J, Ozsarfati J, Kapur BM, Bagli D. Association of in utero exposure to polybrominated diphenyl ethers with the risk of hypospadias. JAMA Pediatr. 2018;172(9):851–6.
38. Luan M, Liang H, Yang F, Yuan W, Chen A, Liu X, Ji H, Wen S, Miao M. Prenatal polybrominated diphenyl ethers exposure and anogenital distance in boys from a Shanghai birth cohort. Int J Hyg Environ Health. 2019;222(3):513–23.
39. Herbstman JB, Sjodin A, Kurzon M, Lederman SA, Jones RS, Rauh V, Needham LL, Tang D, Niedzwiecki M, Wang RY, Perera F. Prenatal exposure to PBDEs and neurodevelopment. Environ Health Perspect. 2010;118(5):712–9.
40. Kim S, Eom S, Kim HJ, Lee JJ, Choi G, Choi S, Kim S, Kim SY, Cho G, Kim YD, Suh E, Kim SK, Kim S, Kim GH, Moon HB, Park J, Kim S, Choi K, Eun SH. 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. 2018;624:377–84.
41. Ding G, Yu J, Cui C, Chen L, Gao Y, Wang C, Zhou Y, Tian Y. Association between prenatal exposure to polybrominated diphenyl ethers and young children's neurodevelopment in China. Environ Res. 2015;142:104–11.
42. Cowell WJ, Lederman SA, Sjodin A, Jones R, Wang S, Perera FP, Wang R, Rauh VA, Herbstman JB. Prenatal exposure to polybrominated diphenyl ethers and child attention problems at 3–7 years. Neurotoxicol Teratol. 2015;52:143–50.
43. Chao HR, Tsou TC, Huang HL, Chang-Chien GP. Levels of breast milk PBDEs from southern Taiwan and their potential impact on neurodevelopment. Pediatr Res. 2011;70(6):596–600.
44. Gascon M, Vrijheid M, Martinez D, Forns J, Grimalt JO, Torrent M, Sunyer J. Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age. Environ Int. 2011;37(3):605–11.

45. Kicinski M, Viaene MK, Den Hond E, Schoeters G, Covaci A, Dirtu AC, Nelen V, Bruckers L, Croes K, Sioen I, Baeyens W, Van Larebeke N, Nawrot TS. Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: a cross-sectional study. Environ Health. 2012;11:86.
46. Eskenazi B, Chevrier J, Rauch SA, Kogut K, Harley KG, Johnson C, Trujillo C, Sjodin A, Bradman A. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. Environ Health Perspect. 2013;121(2): 257–62.
47. Braun JM, Kalkbrenner AE, Just AC, Yolton K, Calafat AM, Sjodin A, Hauser R, Webster GM, Chen A, Lanphear BP. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environ Health Perspect. 2014;122(5):513–20.
48. Chen A, Yolton K, Rauch SA, Webster GM, Hornung R, Sjodin A, Dietrich KN, Lanphear BP. Prenatal polybrominated diphenyl ether exposures and neurodevelopment in U.S. children through 5 years of age: the HOME study. Environ Health Perspect. 2014;122(8):856–62.
49. Vuong AM, Yolton K, Poston KL, Xie C, Webster GM, Sjodin A, Braun JM, Dietrich KN, Lanphear BP, Chen A. Childhood polybrominated diphenyl ether (PBDE) exposure and executive function in children in the HOME study. Int J Hyg Environ Health. 2018;221(1): 87–94.
50. Chao HR, Shy CG, Wang SL, Chen SC, Koh TW, Chen FA, Chang-Chien GP, Tsou TC. Impact of non-occupational exposure to polybrominated diphenyl ethers on menstruation characteristics of reproductive-age females. Environ Int. 2010;36(7):728–35.
51. Windham GC, Pinney SM, Voss RW, Sjodin A, Biro FM, Greenspan LC, Stewart S, Hiatt RA, Kushi LH. Brominated flame retardants and other persistent organohalogenated compounds in relation to timing of puberty in a longitudinal study of girls. Environ Health Perspect. 2015;123(10):1046–52.
52. Johnson PI, Stapleton HM, Mukherjee B, Hauser R, Meeker JD. Associations between brominated flame retardants in house dust and hormone levels in men. Sci Total Environ. 2013;445-446:177–84.
53. Berbel P, Mestre JL, Santamaria A, Palazon I, Franco A, Graells M, Gonzalez-Torga A, de Escobar GM. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid. 2009;19(5):511–9.
54. Idris I, Srinivasan R, Simm A, Page RC. Maternal hypothyroidism in early and late gestation: effects on neonatal and obstetric outcome. Clin Endocrinol. 2005;63(5):560–5.
55. Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 1999;341(8):549–55.
56. Bloom M, Spliethoff H, Vena J, Shaver S, Addink R, Eadon G. Environmental exposure to PBDEs and thyroid function among New York anglers. Environ Toxicol Pharmacol. 2008;25(3):386–92.
57. Turyk ME, Persky VW, Imm P, Knobeloch L, Chatterton R, Anderson HA. Hormone disruption by PBDEs in adult male sport fish consumers. Environ Health Perspect. 2008;116(12):1635–41.
58. Yuan J, Chen L, Chen D, Guo H, Bi X, Ju Y, Jiang P, Shi J, Yu Z, Yang J, Li L, Jiang Q, Sheng G, Fu J, Wu T, Chen X. Elevated serum polybrominated diphenyl ethers and thyroid-stimulating hormone associated with lymphocytic micronuclei in Chinese workers from an E-waste dismantling site. Environ Sci Technol. 2008;42(6):2195–200.
59. Oulhote Y, Chevrier J, Bouchard MF. Exposure to polybrominated diphenyl ethers (PBDEs) and hypothyroidism in Canadian women. J Clin Endocrinol Metab. 2016;101(2):590–8.
60. Wang H, Zhang Y, Liu Q, Wang F, Nie J, Qian Y. Examining the relationship between brominated flame retardants (BFR) exposure and changes of thyroid hormone levels around e-waste dismantling sites. Int J Hyg Environ Health. 2010;213(5):369–80.

61. Dallaire R, Dewailly E, Pereg D, Dery S, Ayotte P. Thyroid function and plasma concentrations of polyhalogenated compounds in Inuit adults. Environ Health Perspect. 2009;117(9):1380–6.
62. Chevrier J, Harley KG, Bradman A, Gharbi M, Sjodin A, Eskenazi B. Polybrominated diphenyl ether (PBDE) flame retardants and thyroid hormone during pregnancy. Environ Health Perspect. 2010;118(10):1444–9.
63. Stapleton HM, Eagle S, Anthopolos R, Wolkin A, Miranda ML. Associations between polybrominated diphenyl ether (PBDE) flame retardants, phenolic metabolites, and thyroid hormones during pregnancy. Environ Health Perspect. 2011;119(10):1454–9.
64. Vuong AM, Webster GM, Romano ME, Braun JM, Zoeller RT, Hoofnagle AN, Sjodin A, Yolton K, Lanphear BP, Chen A. Maternal polybrominated diphenyl ether (PBDE) exposure and thyroid hormones in maternal and cord sera: the HOME study, Cincinnati, USA. Environ Health Perspect. 2015;123(10):1079–85.
65. Abdelouahab N, Langlois MF, Lavoie L, Corbin F, Pasquier JC, Takser L. Maternal and cord-blood thyroid hormone levels and exposure to polybrominated diphenyl ethers and polychlorinated biphenyls during early pregnancy. Am J Epidemiol. 2013;178(5):701–13.
66. Kim S, Park J, Kim HJ, Lee JJ, Choi G, Choi S, Kim S, Kim SY, Moon HB, Kim S, Choi K. Association between several persistent organic pollutants and thyroid hormone levels in serum among the pregnant women of Korea. Environ Int. 2013;59:442–8.
67. Herbstman JB, Sjodin A, Apelberg BJ, Witter FR, Halden RU, Patterson DG, Panny SR, Needham LL, Goldman LR. Birth delivery mode modifies the associations between prenatal polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) and neonatal thyroid hormone levels. Environ Health Perspect. 2008;116(10):1376–82.
68. Chevrier J, Harley KG, Bradman A, Sjodin A, Eskenazi B. Prenatal exposure to polybrominated diphenyl ether flame retardants and neonatal thyroid-stimulating hormone levels in the CHAMACOS study. Am J Epidemiol. 2011;174(10):1166–74.
69. Lin SM, Chen FA, Huang YF, Hsing LL, Chen LL, Wu LS, Liu TS, Chang-Chien GP, Chen KC, Chao HR. Negative associations between PBDE levels and thyroid hormones in cord blood. Int J Hyg Environ Health. 2011;214(2):115–20.
70. Kim S, Park J, Kim HJ, Lee JJ, Choi G, Choi S, Kim S, Kim SY, Moon HB, Kim S, Choi K. Association between several persistent organic pollutants and thyroid hormone levels in cord blood serum and bloodspot of the newborn infants of Korea. PLoS One. 2015;10(5):e0125213.
71. Guo LC, Xiao J, Zhang Y, Yu S, Lin H, Su G, Liu T, Li X, Lv S, Rutherford S, Ma W. Association between serum polybrominated diphenyl ethers, new flame retardants and thyroid hormone levels for school students near a petrochemical complex, South China. Chemosphere. 2018;202:476–82.
72. Vasudevan N, Pfaff D. Molecular mechanisms of crosstalk between thyroid hormones and estrogens. Curr Opin Endocrinol Diabetes. 2005;12(5):381–8.
73. Allen JG, Gale S, Zoeller RT, Spengler JD, Birnbaum L, McNeely E. PBDE flame retardants, thyroid disease, and menopausal status in U.S. women. Environ Health. 2016;15(1):60.
74. Kodavanti PR, Coburn CG, Moser VC, MacPhail RC, Fenton SE, Stoker TE, Rayner JL, Kannan K, Birnbaum LS. Developmental exposure to a commercial PBDE mixture, DE-71: neurobehavioral, hormonal, and reproductive effects. Toxicol Sci. 2010;116(1):297–312.
75. Zhou T, Ross DG, DeVito MJ, Crofton KM. Effects of short-term in vivo exposure to polybrominated diphenyl ethers on thyroid hormones and hepatic enzyme activities in weanling rats. Toxicol Sci. 2001;61(1):76–82.
76. Meerts IA, van Zanden JJ, Luijks EA, van Leeuwen-Bol I, Marsh G, Jakobsson E, Bergman A, Brouwer A. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicol Sci. 2000;56(1):95–104.
77. Ren XM, Guo LH. Molecular toxicology of polybrominated diphenyl ethers: nuclear hormone receptor mediated pathways. Environ Sci Process Impacts. 2013;15(4):702–8.
78. Lim JS, Lee DH, Jacobs DR Jr. Association of brominated flame retardants with diabetes and metabolic syndrome in the U.S. population, 2003-2004. Diabetes Care. 2008;31(9):1802–7.

79. Warner M, Rauch S, Coker ES, Harley K, Kogut K, Sjodin A, Eskenazi B. Obesity in relation to serum persistent organic pollutant concentrations in CHAMACOS women. Environ Epidemiol. 2018;2(4):e032.
80. Ongono JS, Dow C, Gambaretti J, Severi G, Boutron-Ruault MC, Bonnet F, Fagherazzi G, Mancini FR. Dietary exposure to brominated flame retardants and risk of type 2 diabetes in the French E3N cohort. Environ Int. 2019;123:54–60.
81. Erkin-Cakmak A, Harley KG, Chevrier J, Bradman A, Kogut K, Huen K, Eskenazi B. In utero and childhood polybrominated diphenyl ether exposures and body mass at age 7 years: the CHAMACOS study. Environ Health Perspect. 2015;123(6):636–42.
82. Vuong AM, Braun JM, Sjodin A, Webster GM, Yolton K, Lanphear BP, Chen A. Prenatal polybrominated diphenyl ether exposure and body mass index in children up to 8 years of age. Environ Health Perspect. 2016;124(12):1891–7.
83. Vuong AM, Braun JM, Wang Z, Yolton K, Xie C, Sjodin A, Webster GM, Lanphear BP, Chen A. Exposure to polybrominated diphenyl ethers (PBDEs) during childhood and adiposity measures at age 8 years. Environ Int. 2019;123:148–55.
84. Malarvannan G, Van Hoorenbeeck K, Deguchtenaere A, Verhulst SL, Dirinck E, Van Gaal L, Jorens PG, Covaci A. Dynamics of persistent organic pollutants in obese adolescents during weight loss. Environ Int. 2018;110:80–7.
85. Staskal DF, Hakk H, Bauer D, Diliberto JJ, Birnbaum LS. Toxicokinetics of polybrominated diphenyl ether congeners 47, 99, 100, and 153 in mice. Toxicol Sci. 2006;94(1):28–37.
86. Fernie KJ, Laird Shutt J, Ritchie IJ, Letcher RJ, Drouillard K, Bird DM. Changes in the growth, but not the survival, of American kestrels (*Falco sparverius*) exposed to environmentally relevant polybrominated diphenyl ethers. J Toxicol Environ Health A. 2006;69(16):1541–54.
87. Kamstra JH, Hruba E, Blumberg B, Janesick A, Mandrup S, Hamers T, Legler J. Transcriptional and epigenetic mechanisms underlying enhanced in vitro adipocyte differentiation by the brominated flame retardant BDE-47. Environ Sci Technol. 2014;48(7):4110–9.

Chapter 15
Phthalates

Hui-Ju Wen, Han-Bin Huang, Tsung-Lin Tsai, and Shu-Li Wang

Abstract Phthalate esters (PAE) are widely used plasticizers and solvents that are added to many consumer products used in our daily life. PAEs are not chemically bound to the polymer, and therefore are easily released or migrate into the environment including air, drink, foodstuffs, or furniture. Humans can be exposed to PAE through inhalation, ingestion, dermal absorption, or contact with medical devices.

Approximately 70% of the oral dose is excreted in urine. Urinary monoesters are the major urinary metabolites of PAEs and commonly used as internal exposure indexes. Infants and children are more prone to expose to certain PAE than adolescents and adults due to their hand-to-mouth behavior. Adverse effects of PAE exposure have been observed in human studies, and verified in animal experiments. Prenatal PAE exposure is related to decreased levels of testosterone (TT), free TT, progesterone, triiodothyronine, and thyroxine in children. PAE exposure is also associated with developing allergic disease and obesity, decreasing intelligence quotient scores, and affecting psychological behaviors and renal function in children. Higher PAE exposure is also associated with endometriosis, leiomyoma, spontaneous abortion, fertility, and breast cancer in women and semen quality in men. More data are necessary for cancers of the breast, endometrial tissue, ovary, and/or prostate to understand the potential risk related to sex hormone sensitive neoplasms.

In summary, phthalates exposure was found to be associated with various adverse effects related to altered functions of systems including the endocrine, immune, nervous, and reproduction, particularly at critical development windows during fetal, fast growing, and pubertal stages. Future research is directed to multiple generation approach in humans and/or testing animals, and considerations of psychological parameters (i.e., stress) to provide a further wide observational window for the conclusion.

Keywords Phthalate · Epidemiological study · Health effect · Children Environmental health

H.-J. Wen · T.-L. Tsai · S.-L. Wang (✉)
National Institute of Environmental Health Sciences, National Health Research Institutes, Miaoli, Taiwan
e-mail: slwang@nhri.edu.tw

H.-B. Huang
School of Public Health, National Defense Medical Center, Taipei, Taiwan

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_15

15.1 Introduction

H. J. Wen and S. L. Wang

Phthalate esters are widely used plasticizers and solvents that are added to many consumer products, including polyvinyl chloride (PVC) products, cosmetics, food packaging, insecticides, pharmaceuticals, medical items, and construction materials, during their manufacture [1, 2]. Among them, di-2-ethyl-hexyl phthalate (DEHP) and benzyl butyl phthalate (BBzP) are the most commonly used phthalates and they are found in indoor settings (i.e., PVC flooring) [3]. Some phthalates including dibutyl phthalate (DBP), diethyl phthalate (DEP), and dimethyl phthalate (DMP) are used as solvents in cosmetics and personal care products. The chemical structures of phthalate esters contain reacting phthalic anhydride (Fig. 15.1) with alcohol(s) with different chain length as shown in Table 15.1. Phthalate esters are not chemically bound to the polymer and, therefore are easily released, evaporated, and can migrate into *air*, *dust*, *drink*, *foodstuffs*, *furniture*, and related products [2]. This chapter primarily focuses on the findings of studies on the exposure and related health effects particularly with tentative specific hypotheses in the general population. Good study designs for causal inference such as those with prospective cohort follow-up approaches are prioritized.

15.2 Metabolism of Phthalates

H. B. Huang

Following absorption, phthalates are rapidly metabolized by hydrolysis and subsequent oxidation reactions in the human body. The content of phthalate metabolites in the human urine represents a measure of the exposure to the respective parent phthalate that occurred within the last 24 h [4]. Human metabolism studies have shown that the simple monoesters are the major urinary metabolites of short-chain phthalates such as di-*n*-butyl phthalate (DnBP), diisobutyl phthalate (DiBP), or BBzP. Approximately 70% of the oral dose is excreted in urine [5]. In the case of long-chain phthalates such as DEHP, diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), and dipropylheptyl phthalate (DPHP), most of the simple monoester is further metabolized to a number of oxidative metabolites.

Fig. 15.1 General chemical structure of an *o*-phthalate

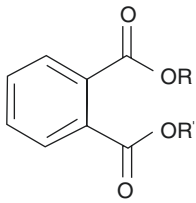
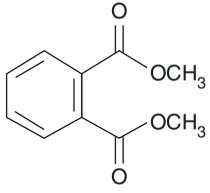
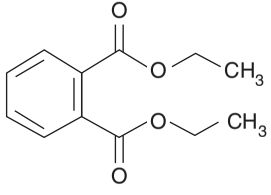
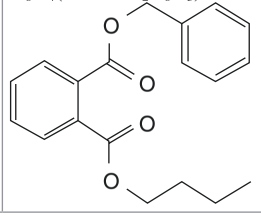
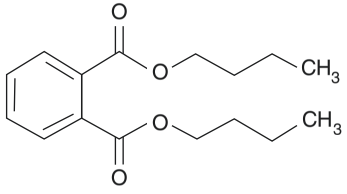
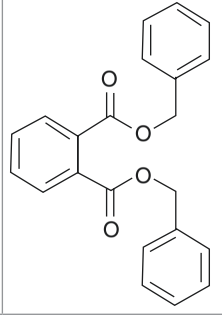


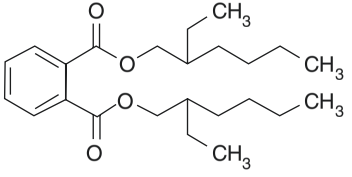
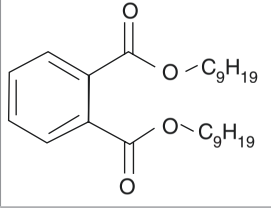
Table 15.1 Chemical structure, metabolites, and possible source of commonly studied phthalates

Parental phthalates	Structural formula	Molecular weight (g/mol)	Urinary metabolites	Possible source	Acceptable exposure level (μg/[kg bw·day])
Dimethyl phthalate (DMP)	$C_6H_4(COOCH_3)_2$ 	194.18	Monomethyl phthalate (MMP)	Personal care products, fragrance, insecticides, adhesives	–
Diethyl phthalate (DEP)	$C_6H_4(COOC_2H_5)_2$ 	222.24	Monoethyl phthalate (MEP)	Personal care products, fragrance	800 (U.S.-EPA)
Butyl benzyl phthalate (BBzP)	$C_6H_4(COOCH_2C_6H_5)$ 	312.36	Monobenzyl phthalate (MBzP)	Vinyl foams, artificial leather, food packages	500 (EFSA) 200 (U.S.-EPA)

(continued)

Table 15.1 (continued)

Parental phthalates	Structural formula	Molecular weight (g/mol)	Urinary metabolites	Possible source	Acceptable exposure level (μg/[kg bw·day])
Dibutyl phthalate (DBP)	$C_6H_4[COO(CH_2)_3CH_3]_2$ 	278.34	Mono-*n*-butyl phthalate (MnBP) Mono-*iso*-butyl phthalate (MiBP)	Food packages, adhesives, and cosmetics, personal care products, medications	10 (EFSA) 100 (U.S.-EPA)
Dibenzyl phthalate (DBzP)	$C_6H_4(COOC_2H_2C_6H_5)_2$ 	346.38	Monobenzyl phthalate (MBzP)	Vinyl flooring, adhesives, sealants, food packaging, furniture, and artificial leather	–

Di-2-ethylhexyl phthalate (DEHP)	$C_6H_4[COOCH_2CH(C_2H_5)(CH_2)_3CH_3]_2$ 	390.56	Mono-(2-ethylhexyl) phthalate (MEHP)	PVC-containing product, flexible plastics, medical devices, food package, textile	50 (EFSA) 20 (U.S.-EPA)
			Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)		
			Mono-(2-ethyl-5-oxylhexyl) phthalate (MEOHP)		
			Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)		
			Mono(2-carboxymethylhexyl) phthalate (MCMHP)		
Diisononyl phthalate (DiNP)	$C_6H_4[COO(CH_2)_6CH(CH_3)_2]_2$ 	418.61	Mono-*iso*-nonyl phthalate (MiNP)	PVC, flexible plastics	150

EFSA European Food Safety Authority, *U.S.-EPA* United States Environmental Protection Agency

Only between 2 and 7% of the dose of these long-chain phthalates is excreted as the simple monoester. The secondary oxidized metabolites are the main metabolites excreted in human urine (Table 15.1) [6–10]. For example, secondary oxidized DEHP metabolites such as mono- (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl)phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), and mono- [2-(carboxy-methyl)hexyl]phthalate (MCMHP) represent the major share of DEHP metabolites excreted in urine [11].

15.3 Phthalate Exposure Assessment

H. J. Wen

Phthalate esters are widely used in daily life and the environment (Table 15.1). Exposure to phthalate occurs through ingestion, inhalation, dermal absorption, or contact with medical devices [2]. It can cross the placenta and, thus, has a detrimental health effect on the fetus and newborns. Phthalate esters can be detected in samples of dust, food stuff, water, soil, and breast milk. *Moreover, the predominant phthalate exposure may be inconsistent in different area*. For example, a study comparing the levels of phthalate in indoor dust reported that levels of DEP, DEHP, and BBzP measured in dust collected from the USA were approximately 5–10 times higher than those from China. Among measured phthalates in indoor dust, DEHP, BBzP, and *DnBP* were the predominate compounds in the USA, whereas those in Chinese cities were DEHP, *DnBP*, and DiBP [12].

Urinary concentrations of phthalate metabolites are commonly used biomarker for phthalate exposure. A study comparing urinary concentrations of 14 phthalate metabolites in a general population aged 21–49 years from seven countries reported that the highest median level of Σ_{14} metabolites was from Kuwait (1050 ng/mL) followed by India (398 ng/mL), China (234 ng/mL), Vietnam (133 ng/mL), Japan (120 ng/mL), Korea (117 ng/mL), and Malaysia (95 ng/mL). Among urinary phthalate metabolites, Σ_5MEHP was the predominate metabolite in Vietnam, Japan, Korea, and Malaysia; Σ_5MEHP, MiBP, and MBP were predominant metabolites in China; and Σ_5MEHP and MEP were predominant in Kuwait and India [13]. In Taiwan, the predominate phthalate metabolite (urinary creatinine adjusted) was MMP followed by MECPP and MEHHP. Moreover, a higher concentration of MEHP and lower concentrations of MEP, MnBP, MiBP, and MBzP were found in a general population (≥7 years) from Taiwan than that from the USA (>6 years) or Canada (19–49 years) [14]. For children, lower estimated DEHP daily intake levels (Table 15.2) were observed in European countries than in Taiwan [15].

Because of their hand-to-mouth behavior, infants and children are more prone to exposure to certain phthalates than adolescents and adults are [16]. A birth cohort study in central Taiwan shown that the geometric mean of the estimated daily intake in pregnant women was as low as 0.02 for *BBzP* to as high as 2 (μg/[kg bw·day]) for DEHP, whereas in children aged 2–3 years it was 0.17 for *BBzP* to 8.1

Table 15.2 Formula for calculating estimated daily intake levels using di-2-ethyl-hexyl phthalate (DEHP) as an example [174]

$$\text{Estimated daily intake levels (μg/[kg bw·day])} = \frac{\text{UE}_{\text{sum}}(\text{μmol/g}) \times \text{CE}(\text{g/day})}{F_{\text{UE}} \times \text{BW}(\text{kg})} \times \text{MW}_{\text{DEHP}}$$

UE_{sum}: Sum of three urinary creatinine-adjusted DEHP metabolite concentrations;
CE: Sex-specific body height-based reference values for urinary creatinine excretion;
F_{UE}: Molar fraction of excreted metabolite relative to total intake at 24-h post-dosing;
BW: Body weight;
MW_{DEHP}: Molecular weight of DEHP.

(μg/[kg bw·day]) for DEHP, and in children aged 5–6 years it was 0.17 for *BBzP* to 10.9 (μg/[kg bw·day]) for DEHP, respectively [17]. Daily intake levels of DnBP, DiBP, BBzP, and DEHP decreased with increasing age, which were also observed in an Austrian study [18] *and a Japan study* [19]. Moreover, children aged 7–18 years had significantly higher urinary levels of MEOHP, MECPP, and MnBP than the adult population did in a nationwide survey in Taiwan [14].

In May 2011, phthalate-tainted foodstuffs were discovered in Taiwan when DEHP and diisononyl phthalate (DiNP) were illegally added to foodstuffs as a clouding agent to replace palm oil [20]. Children are suspected to be exposed to higher phthalate levels before the age of 12 years, and the median estimated DEHP daily intake level is 29.65 and 25.85 (μg/[kg bw·day]) for boys and girls, respectively, according to a calculation using exposure assessment questionnaire and urinary metabolite concentrations. Specifically, the DEHP exposure level of 26.0% of children exceeded that of the European Food Safety Authority (EFSA) guideline (50 μg/[kg bw·day]) [15]. However, the urinary concentration of DEHP metabolites in children investigated immediately after the incident was reduced to background levels 6 months after consumption of the contaminated food was discontinued [21]. This result suggests that DEHP exposure can be effectively reduced by avoiding the use of phthalate-containing product.

Although the exposure level of phthalate is still lower than the accepted limit in daily life (Table 15.1), adverse effects of low-dose phthalate have still been observed in animal and human studies [15, 22–24]. *A more recent report revealed a further reduced maternal total daily intake was suggested that in order to protect fetus from future behavioral problems after birth* [25]. In the next section, we will briefly introduce the health impact of phthalate exposure.

15.4 Health Effects of Phthalate Exposure (Table 15.3)

Compared with adults, children tend to be more vulnerable to environmental pollutants such as phthalate due to the fast development of their various systems. Therefore, we will focus more on the health effect of phthalate exposure in children in this section.

Table 15.3 Summary of potential health effects of prenatal and postnatal phthalate exposure

Health status	DMP	DEP	DBP (DnBP, DiBP)	DBzP	DEHP (Others)
Sex hormone and reproductive development	TT ↓ Free TT ↓ PG ↓ E2 ↓	Delayed pubertal development		TT ↓ Free TT ↓ PG ↓ SHBG ↑ Delayed pubertal development	TT ↓ Free TT ↓ FSH ↑ SHBG ↑ E2 ↓ Sperm quality ↓ Uterus size ↓ Delayed pubertal development
Genital development		AGI ↓	AGI ↓ ASD ↑ Male genital abnormality↑(paternal exp.)	AGI ↓ ASD ↑	AGD ↓ Male genital abnormality↑
Thyroid function		Free T_4 ↓ T_3 ↓ Free T_3 ↓		Free T_4 ↓ TSH ↓	T_3 ↓ T_4 ↓ TSH ↓ Free T_4/T_4 ratio ↓
Neurocognitive and behavioral development			MDI ↓ PDI ↓ IQ ↓	MDI ↓ PDI ↓	MDI ↓ PDI ↓ IQ ↓ Externalizing behavior score ↑
Allergic diseases		Wheezing ↑ Asthma ↑	Atopic dermatitis↑	Asthma ↑ Atopic dermatitis↑ Th_2 immune response ↑	Asthma ↑ Wheezing ↑ Atopic dermatitis↑ IgE ↑ IL-4 ↑ INF-γ ↓

Obesity		WC ↑ BMI ↑ Overweight ↑ Obesity ↑ Abdominal obesity ↑	WC ↑ BMI ↑ Overweight ↑ Obesity ↑ Abdominal obesity ↑	WC ↑ BMI ↑	Adipogenesis ↑ PPARα expression ↑ Leptin ↑ Adiponectin ↓ Fatty cells ↑ WC ↑ BMI ↑ Abdominal obesity ↑
Renal function		Kidney weight ↑	Kidney weight ↑ Kidney size ↓ Renal lesions ↑		Albumin/creatinine ratio ↑ Microalbuminuria ↑
Gynecologic health	Semen quality ↓ (adult exp.)	Semen quality ↓ (adult)	Semen quality ↓ (adult)	Semen quality ↓ (adult)	Semen quality ↓ (adult)
	Fecundity ↓ (male exp.)	Fecundity ↓		Fecundity ↓ (male)	Fecundity ↓
			Endometriosis ↑	Endometriosis ↑	Endometriosis ↑
			Ovarian function ↓	Ovarian function ↓	Ovarian function ↓
	Leiomyoma ↑		Pregnancy loss ↑		Leiomyoma ↑
Cancer		Breast CA ↑ (Mexico) Breast density ↑	Breast CA ↑ (paternal exp.)	Breast CA ↓ (Mexico)	Breast CA ↑ (Alaska, NY leans)

DMP dimethyl phthalate, *DEP* diethyl phthalate, *DBP* dibutyl phthalate, *DBzP* dibenzyl phthalate, *DEHP* di-2-ethylhexyl phthalate, *TT* testosterone, *E2* estradiol, *PG* progesterone, *SHBG* sex hormone-binding globulin, *FSH* follicle-stimulating hormone, T_3 triiodothyronine, T_4 thyroxine, *TSH* thyroid-stimulating hormone, *MDI* mental developmental index, *PDI* psychomotor developmental index, *IQ* intelligence quotient, *PPAR* peroxisome proliferator-activated receptor, *BMI* body mass index, *WC* waist circumference

15.4.1 Sex Hormones and Reproductive Health

H. J. Wen and S. L. Wang

Phthalate esters are *well-known environmental endocrine disruptor and* reported to have anti-androgenic and weak estrogenic effects. DEHP has been associated with decreased testosterone (TT) levels in young, male adults [26]. In an 11-year follow-up birth cohort study in Taiwan [27], prenatal *BBzP* and DEHP exposure was related to decreased levels of TT, free TT, and progesterone (PG) in girls. Moreover, childhood *BBzP*, DMP, and DEHP exposure was associated with decreased levels of TT, free TT, estradiol (E2), and PG in children aged 2–11 years [27]. In the same cohort, maternal DEHP exposure was also associated with reduced free TT and E2 levels in neonatal girls [28]. Consistently, a negative effect of cord blood TT/E2 ratio was found in newborns with higher maternal DEHP exposure in a Japanese birth cohort study [29]. Among affected children with suspected exposure to higher DEHP in the Taiwan episode, significantly higher concentrations of follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG) were found in girls >12 years old [15]. A positive association with E1 and E2 levels was reported in pregnant women with higher urinary DEHP metabolites, which might subsequently be linked to genital abnormalities in neonatal boys [30]. In male PVC workers, higher E2 levels and elevated E2/TT ratios were also found in those identified with higher urinary DEHP metabolite concentrations [31]. In addition, an anti-estrogenic effect was also reported in that study [31]. A negative association was found between childhood DEHP exposure and E2 levels [27].

Phthalate ester exposure was also associated with childhood reproductive system development. Swan et al. [32] firstly used a rodent model and then reported the inverse association between anogenital distance (AGD)/bw and prenatal exposure to MEP, MBzP, and MiBP in 2- to 36-month-old boys [32]. This research group further found that first trimester exposure to DEHP was associated with increased risk of genital abnormalities including hypospadias, undescended testes, and isolated hydrocele [33]. Prenatal exposure to DEHP was also found to be inversely associated with male AGD [34] at birth, particularly for first trimester exposure [35]. Wenzel et al. [36] established a similar negative association in boys, and a positive association for female infants between the anoscrotal distance (ASD) and prenatal exposure to MBP and BBzP with racial difference consideration.

In utero exposure to DEHP was also associated with a shorter AGD in boys [37] and a lower sperm volume in adolescent boys [38]. In addition, prenatal DEHP exposure was significantly related to reduce uterus size in girls aged 8 and 11 years [39] and delayed breast development in girls aged 8 and 13 years [40]. Prenatal DBzP exposure was also linked to delayed pubic hair development and higher SHBG levels in boys aged 8–14 years [41]. Pre-pubertal exposed to DEHP, DnBP, DBzP, and DEP was associated with delayed pubertal development in children, especially girls [42]. In animal studies, an adverse effect of DEHP exposure on Leydig cells was found and subsequently linked to decreased TT concentration, reduced sperm counts, and abnormal testicular development [43, 44].

These observations could be associated with subsequent effects on reproductive function in adulthood.

The balance of sex steroid hormone levels in the somatic nervous system is controlled and regulated by the hypothalamus-pituitary-gonadal (HPG) axis through a negative feedback mechanism. Phthalate esters are well-known environmental endocrine disruptor that may interfere with the normal function of the HPG axis. Anti-androgenic and weak estrogenic effects of phthalate exposure induce an imbalance in the levels of reproductive hormones in the body, with potentially adverse effects on reproductive system development. The anti-androgenic effect of MEHP appears to be related to non-receptor-mediated mechanisms such as protein synthesis inhibition, disruption of β-galactosidase gene transcription, and enzyme inhibition whereas the anti-estrogenic effect of MEHP was caused by direct-receptor activity at high concentrations [45].

15.4.2 Thyroid Function

H. B. Huang and S. L. Wang

Thyroid hormones are essential for proper cell differentiation and development in the human body, particularly in fetuses and newborns. Information on the relationship between exposure to phthalates and thyroid function in birth cohort studies is limited. Kuo et al. [46] indicated that maternal MBzP levels were negatively associated with serum thyroid-stimulating hormone (TSH) in cord blood. *Wu et al.* [47] *reported that serum TSH levels decreased when the children were exposed to high concentrations of DEHP-tainted foodstuffs (>500 ppm of DEHP in affected food items as the high exposure group) and that triiodothyronine (T3) may be partially recovered after stopping exposure*. Morgenstern et al. [48] found positive associations between maternal MEHP levels and free thyroxine (T_4) in 3-year-old children.

Huang et al. [22] reported that the T4 levels *from children at 2–8 years of age* were inversely associated with maternal urinary MEHHP and MEOHP in boys, and free T4 levels were inversely associated with levels of maternal urinary MEP, MBzP, and urinary MEHP in girls. Two prospective cohort studies did not find any associations between prenatal exposure to phthalate and thyroid hormone levels in newborns or infants [49, 50]. A cross-sectional study of 845 children aged 4–9 years old in Denmark determined that T_3 and free T_3 levels were negatively correlated with crude phthalate concentrations (*without the urinary creatinine corrected*), including MEP and DEHP metabolites [51]. Furthermore, cross-sectional studies have reported inverse relationships between exposure to DEHP metabolites and free T_4/T_4 in adults [52, 53].

Previous studies have shown that exposure to phthalates during gestation could influence the levels of thyroid hormone in pregnant women [54, 55], and indicated that the timing of phthalate exposure may be an important determinant of susceptibility to thyroid disruption in pregnant women. *Because each organ system has a different developmental trajectory and the sensitive window for exposure to cause*

toxicity varies during tissue development in pregnancy, the effects of in utero exposure depend not only on the type and dose of the chemical but also on the exposure time [56]. Although exposure to phthalate could affect thyroid hormones homeostasis in pregnant women, children, and adults, large-scale prospective cohort studies are required to examine these associations between both prenatal and postnatal exposure to phthalates and thyroid function in children, and determine the trimester during which exposure to phthalates is crucial to homeostasis of fetal thyroid hormones.

Biological mechanisms underlying the thyroid disruption effects of phthalate exposure have been examined in vivo and in vitro studies. Several studies have reported thyroid alterations and lower plasma T4 concentrations in rats fed with DEHP-contaminated products than in controls [57, 58]. In vitro studies have indicated that DEHP acts as a thyroid receptor antagonist [59, 60], and causes changes in iodide uptake by altering the sodium–iodide symporter [61]. Moreover, phthalates can influence thyroid hormones not only through biosynthesis and bio-transport but also through biotransformation and metabolism [62]. Recent animal experiments have suggested that DEHP can influence thyroid hormones by disturbing the hypothalamus-pituitary-thyroid axis and activating the Ras-Akt-thyrotropin-releasing hormone receptor pathway and inducing hepatic enzymes [63]. DEHP can also disrupt thyroid function, including decreased T4, by damaging thyroid follicles and affecting TTF-1, PAX8, NIS, TPO and the deiodinase protein family [64].

Although exposure to phthalate could affect thyroid hormones homeostasis in pregnant women, children, and adults, large-scale prospective cohort studies are required to examine these associations between both prenatal and postnatal exposure to phthalates and thyroid function in children, and determine the trimester during which exposure to phthalates is crucial to homeostasis of fetal thyroid hormones.

15.4.3 Neurocognitive and Behavioral Development

H. B. Huang and S. L. Wang

The developing human brain is vulnerable to toxic chemical exposures including endocrine disruptors [65]. The major windows of developmental vulnerability are in utero, infancy, and early childhood [66]. Because of the widespread use of phthalates, concerns have been raised on the adverse health effects of phthalate exposure on children's neurocognitive and development.

Previous prospective cohort studies have reported inverse relationships between exposure to *DnBP*, BBzP and DEHP metabolites in urine during the third trimester in pregnant women and mental development index (MDI)/psychomotor development index (PDI) in children aged 6 months to 3 years [67, 68]. Only two studies have reported negative associations between exposure to phthalates (such as *DnBP*/DEHP metabolites) in pregnant women and MDI in girls aged 24–36 months, and positive relationships between maternal MnBP/MBzP levels in urine and PDI in boys following exposure [69, 70].

Other studies have shown that prenatal exposure to *DnBP* metabolites or postnatal exposure to DEHP metabolites could be associated with decreasing intelligence quotient (IQ) scores of children aged 2–11 years [71–73]. Childhood exposure to phthalates was suggested to *decrease* neurocognitive function in Taiwanese and Korean studies [72, 73]. In fact, most birth cohort studies so far have reported the adverse health effects of prenatal exposure to phthalates on cognitive function in children; only one study did not find any associations between maternal MEHP levels in blood samples and MDI or PDI in children aged 6–18 months [74]. Taken together, these findings indicate that future research should consider the adverse effects of both prenatal and postnatal phthalates exposure on cognitive function in children. Furthermore, sex-specific associations between phthalate exposure and cognitive function would be needed to investigate this phenomenon in future large-scale studies.

Regarding behavioral problems, Engel et al. [75] found that maternal low-molecular-weight phthalate concentrations were positively associated with aggression, attention problems, conduct problems, and depression scores using the Behavior Assessment System for Children (BASC) in 4–9-year-old children. Whyatt et al. [68] found positive associations between maternal MnBP and MBzP levels and scores for withdrawn and internalizing behaviors using the Child Behavior Checklist (CBCL) in 3-year-old children. Kobrosly et al. [76] found that maternal DEHP metabolite concentrations were positively associated with scores for somatic problems among children at 6–10 years of age.

Lien et al. [23] reported positive associations between maternal urinary DEHP metabolite levels and externalizing domain behavioral problem scores in 8-year-old children. Most of these studies reported positive associations between prenatal exposure to phthalates and scores of neurobehavioral development in 1–10-year-old children. However, these studies did not show similar results and whether certain phthalates may affect different domains of neurobehavioral development remains unclear.

Two studies reported conflicting observations of sex-specific associations between phthalate exposure and neurobehavioral development [68, 75]. Other studies have not observed any sex-specific associations between phthalate exposure and internalizing behavior or externalizing behavior scores [23, 76]. Future studies are required to confirm the sex-dependent effects and elucidate the underlying modulating effects of sex.

The mechanisms underlying the adverse effects of phthalates on neurocognitive and neurobehavioral development are unclear. The regulations of neurotransmitter system, the peroxisome proliferator-activated receptors (PPARs), and thyroid function could be suggested possible biological links between phthalate exposure and these adverse outcomes. Phthalates could disturb dopamine receptor D2, tyrosine hydroxylase, and homeostasis of calcium-dependent neurotransmitters, leading to a reduction in the release of dopamine [77–79]. Several studies have observed that ligands of PPAR played roles in lipid metabolism, cellular proliferation, and the inflammatory response [80]. Its signal transduction pathway is related to the progression of neurodegenerative and psychiatric diseases and its relation to cognitive

function [81]. In addition, thyroid hormones play a fundamental role in neurocognitive development and hippocampal function, and hippocampal dysfunction often leads to deficits in learning and memory in rats [82, 83]. The possible biological links between phthalate exposure and thyroid function were descripted in Sect. 15.4.2.

Exposure to phthalates could affect neurocognitive and neurobehavioral development in human studies. However, longitudinal studies need to consider the adverse effects of both prenatal and postnatal exposure on cognitive and behavioral development in children, as well as investigate sex-specific associations.

15.4.4 Allergic Diseases

H. J. Wen and S. L. Wang

Allergic diseases, including atopic dermatitis (AD), asthma, and allergic rhinitis, are common chronic inflammatory diseases in children. The prevalence of allergic diseases in children increased from 1997 to 2003 [84]. Allergic diseases have profound effects on medical cost and quality of life in children and their families because they are chronic and associated with repetitive symptoms [85]. Environmental and genetic factors all contribute to allergic disease development. A recent review has shown that exposure to environmental endocrine disruptors, such as plasticizers, increases the risk of allergic diseases [86].

Higher risk of developing allergic diseases was reported in children residing in homes with PVC plastic flooring than in those with non-PVC flooring [87]. Household dust with high concentrations of DMP, DEHP, DiBP, and BBzP was also associated with AD, conjunctivitis, and allergic rhinitis in children [88]. Allergic patients sensitized to house dust mite and exposed to higher DEHP levels in dust showed altered secretion of inflammatory cytokines, including interleukin (IL)-6, eosinophils, and granulocyte-colony-stimulating factor (G-CSF) in their nasal mucosa [89]. An increased risk of asthma was observed in children in the highest quartiles of mono-carboxynonyl phthalate and mono-carboxyoctyl phthalate exposure in a Norwegian study [90]. In an 8-year longitudinal birth cohort study, phthalate ester was found to be associated with the development of wheezing and asthma in children, particularly for boys exposed to higher DEHP and DEP levels [91]. In a Japan cohort study, maternal MEHP level was associated with increased risks of wheezing and allergies up to 7 years of age [92]. Maternal urinary concentrations of MnBP were also associated with an increased asthma risk in children form a German cohort study [93]. Prenatal exposure to *high-molecular-weight phthalates* (*i.e.*, *DEHP and MBzP*) could also increase the risk of asthma symptoms and respiratory tract infection in a Spanish cohort study of 7-year-old children [94].

AD is a common chronic skin disorder in children. Maternal urinary MiBP concentration was associated with AD in 3-year-old children [95]. *An association was found between childhood BBzP exposure and AD in children aged 2 years* [24]. DEHP exposure was *also* linked with an increased risk of AD in children aged

3 years [96] and higher concentrations of urinary MBP were measured in children with AD than in those without AD [97]. Among children with high MBP or MBzP levels, a higher risk of developing AD was found in those with filaggrin candidate genotype that might show a gene–environment interaction in allergic disease than in those without this genotype [98].

Moreover, DEHP was identified as an adjuvant that promotes allergic asthma. Following exposures to DEHP and allergens, DEHP increased the levels of immunological and inflammatory markers in mice [99]. DEHP and BBzP might promote the T helper 2 (Th_2) immune response to increase sensitization by the stimulation of T-cell responses and suppression of interferon (IFN)-α/IFN-β expression [100]. A recent study also reported an increased risk of allergic airway inflammation in offspring with higher maternal *BBzP* exposure through epigenetic alterations that modulate the expression of genes involved in Th_2 differentiation [93].

Phthalate exposure associated with allergic disease has been well reported. Allergic diseases are thought to be chronic inflammatory disorders. Phthalate exposure that interferes the balance of Th1/Th2 immune response [101] and increases the production of Th2 related cytokines (i.e. IL-4, IL-13) [102–104] and inflammatory biomarkers (i.e., tumor necrosis factor α, eosinophils) [89, 105] is suggested as a potential mechanism for allergic disease occurrence. However, such effect is still needed further investigation.

15.4.5 Obesity

H. J. Wen and S. L. Wang

Being overweight and obesity are major risk factors for cardiovascular diseases (CVD), metabolic syndromes, diabetes, and cancer, and important health issues that need to be addressed for diseases prevention. Recently, age at the onset of CVD has obviously decreased and the prevalence of obesity in children has gradually increased simultaneously. According to a report of the World Health Organization (WHO), more than 41 million children <5 years old were overweight in 2016 [106]. Children who are overweight or obese are likely to stay obese into adulthood and these conditions are related to the development of chronic diseases such as CVD and metabolic syndromes at a younger age than in those who have a normal weight.

Maternal DEHP exposure was related to decreased cord blood leptin levels in newborns [107]. Maternal urinary concentrations of mono-3-carboxypropyl phthalate (MCPP) were positively associated with overweight/obese status in children from three prospective cohort studies in USA [108]. In a cross-sectional study by the National Health and Nutrition Examination Survey (NHANES) in 1999–2002 in the USA, increasing body mass index (BMI) levels and waist circumference (WC) were found in 20- to 59-year-old men with high urinary concentrations of MBzP, MEHHP, MEOHP, MEP, or MBP. Adolescent girls with higher urinary MEP concentration also had higher BMI and WC.

However, an inverse association was found between the BMI and MEHP levels in adolescent girls and women aged 20–59 years [109]. In a Chinese study of 8–15 years old children, a positive association was found between urinary phthalate metabolites and BMI or WC, particularly with MEHP and MEP [110]. A positive dose-response relationship was observed between urinary phthalate metabolite concentrations (particularly, metabolites of DEP, DiBP, DnBP, and DEHP) and the anthropometric indices for abdominal obesity, including WC, waist-to-height ratio, waist-to-hip, and skin fold thickness in 6.5- to 15-year-old children from Taiwan [111].

Low-molecular weight (LMW) phthalate metabolites, including MEP, MBP, and MiBP, were also linked with overweight and obesity in non-Hispanic black children [112]. A 0.8 kg increase in body weight, 1.5% increment in prevalence of obesity, and 3.3% increment in prevalence of overweight were observed in children with LMW phthalate concentration increasing from the 25th to the 75th percentile [112]. Conversely, an inverse association was found between maternal non-DEHP component score (considered with MCPP, MiBP, MBP, MBzP, and MEP) and BMI *z*-score, body fat percentage, BMI, and WC in boys only in a longitudinal follow-up study [113].

In 2006, Drs. Grun and Blumberg proposed an obesogenic hypothesis and considered that endocrine disrupting chemical exposure could induce adipogenesis related to obesity [114]. Phthalate esters are considered obesogenic and are associated with overweight and obesity related to adipogenesis due to active expression of PPARα and PPARγ [115, 116]. Moreover, plasticizer exposure was also reported to induce fat cells to secrete fat hormones (adipokines, such as leptin and adiponectin). An experimental study in female mice fed a 0.05, 5, or 500 (mg/[kg bw·day]) DEHP-containing diet for 8 weeks showed a significant increase in body weight and visceral adipose tissue with larger adipocytes in DEHP-exposed mice compared with the controls.

The increase in PPARα and PPARγ mRNA expression in the liver and leptin mRNA expression in visceral adipose tissue, and the decrease in adiponectin mRNA expression in visceral adipose tissue were also observed in the group exposed to the highest levels of DEHP [117]. A negative effect on adiponectin levels and enhanced weight gain and fat mass were also found in mice fed 0.05 mg/(kg bw·day) DEHP-containing diet for 10 weeks [118].

Overall, phthalates affect the function of PPARα and PPARγ, increase BMI and fat cells, and alter the production of adipokines. However, the effect of phthalate exposure on obesity may differ based on sex and age and further investigation is warranted.

15.4.6 Renal function

T. L. Tsai and S. L. Wang

Studies of kidney health related to phthalate exposure are scarce. A prospective study of 19 newborns exposed to high DEHP levels from extracorporeal membrane oxygenation (ECMO) showed that all participants had normal renal function after laboratory

examination at 14–16 years old [119]. A cross-sectional study of 667 children (6–19 years old) from the National Health and Nutrition Examination Survey (NHANES) from 2009 to 2010 reported that high-molecular-weight (HMW) phthalates exposure was associated with altered renal function, in which a three-fold increase in urinary metabolites of DEHP resulted in a 0.55 mg/g increase in the albumin/creatinine ratio [120]. Moreover, a cross-sectional study of 195 children in Taiwan also found an association between urinary DEHP metabolites and increased urinary albumin/creatinine ratio, and between high DEHP exposure and increased microalbuminuria [121].

In experimental studies, increased relative kidney weights were observed in male and female rats and female mice after exposure to *DBP* by oral administration for 13 weeks, and no kidney lesions were observed in rats [122]. In a study of cynomolgus monkeys, no changes were observed in histological examinations or kidney weights after exposure to DEHP at a dose of 500 mg/(kg bw·day) [123]. Following chronic exposure to DEHP at 354 mg/(kg bw·day) in the diet for 104 weeks, naturally chronic progressive nephropathy occurred in mice with no significant toxicological changes in urinalysis indices [124]. In male mice fed a 0.01 or 0.05% DEHP-containing diet for 22 months, increased production of reactive oxygen species (ROS) in the glomeruli was observed, which may also induce glomerulonephritis [125]. A recent study in which pregnant rats were administered DnBP 850 mg/(kg bw·day) from gestation day 12–18 reported that such exposure may induce renal lesions in offspring. On postnatal day 1, the offspring of the *DnBP*-exposed group had significantly lower bw and less kidney size than the offspring of the unexposed group did [126]. Furthermore, a histopathological examination of the kidney tissue showed renal fibrosis in adult offspring of *DnBP*-exposed group [126]. Although the molecular mechanisms for DEHP-induced renal injury still remain unclarified, a recent experimental study reported exposure to DEHP, but not its metabolite MEHP, induced epithelial-to-mesenchymal transition in renal proximal tubular cells through the up-regulation of AKT pathway and the down-regulation of peroxisome proliferator-activated receptor γ related signaling [127].

After synthesizing the observations of these studies, exposure to phthalates might associate with elevated urinary albumin/creatinine ratio in human, and ROS production and impaired kidney tissue in animal. However, owing to the inconsistent observations of human and animal studies, the association between phthalate exposure and renal function remains unclear and more evidence-based investigations are required.

15.4.7 Reproductive Health and Breast Cancer

S. J. Wang

As shown in Table 15.3, various issues related to the effects of phthalate on gynecological health and cancer, *with less evidence as compared to development effects*, were reviewed up to *July 2019* and are addressed below.

15.4.7.1 Semen Quality

In addition to prenatal exposure effects, current exposure to MMP [128], MEP, MBzP [129], and DEHP [130, 131], and increased MEHP% [132] was also found to be adversely associated with semen quality, assessed using sperm count, concentration, morphology and motility in adults. DEHP may also contribute to the decline in semen quality indicated by the number of abnormal heads [133]. Exposure to phthalate, especially DEHP, may reduce sperm production by inhibiting INSL3 expression levels and testosterone levels in adult testes [130, 134]. Oxidative stress and subsequent decrease in testosterone secretion were the potential underlying mechanism of DBP-induced testicular toxicity [135].

15.4.7.2 Fecundity

Buck et al. [136] found a 20% reduction in fecundity (longer time-to-pregnancy, TTP) associated with DMP and BBzP exposure in men but not women [136]. High exposure to DEP in women was found to be significantly associated with a longer TTP in one study [137] but not in other study [138]. More follow-up studies evaluating both parents are needed for clarification. Currently various reproductive effects including decreased semen quality and women's adverse conditions (i.e., ovarian dysfunction) described below provide mechanisms for the decreased fecundity.

15.4.7.3 Endometriosis and Ovarian Function

Exposure to DEHP, *DnBP*, and BBzP was found to be associated with endometriosis in case–control studies in India [139] and Korea supported by animal models [140, 141]. Furthermore, in Taiwan similarly exposure outcomes were observed where adenomyosis, in particular, enhanced with the glutathione *S*-transferase (GST) M1 null genotype [142]. In addition, decreased ovarian function was associated with increased exposure to DEHP [143, 144], *DnBP*, and BBzP [144].

Phthalate was found to establish endometriosis by enhancing invasive and proliferative activities of endometrial cells, via the increases of matrix metalloproteinase (MMP)-2 and MMP-9 activities, cellular invasiveness, extracellular signal-regulated kinase (Erk) phosphorylation, and p21-activated kinase 4 expression [141]. DEHP at high dose may cause endometriosis through induced oxidative stress. DEHP increased reactive oxygen species (ROS) generation and decreased expression of superoxide dismutase (SOD), glutathione peroxidase (GPX), heme oxygenase (HO), and catalase (CAT) in human endometrial stromal cells [145]. Additionally, p-ERK/p-p38 and NF-κB mediated transcription was increased. Besides, DEHP induced estrogen receptor-α (ER-α) expression in a dose-dependent manner. DEHP

may also induce human aldo-keto reductases (AKR) activity in the endometrium, which might prolong the increased risk of endometriosis [146].

Phthalates disrupt ovarian functions and impact ovarian reserve at various folliculogenesis stages. Exposure to MEHP in pregnant mice led to premature ovarian senescence in the F1 generation, and depletion of the primordial-follicle pool in the F1 and F2 generations, via acceleration in follicular recruitment [147]. Altered DNA methylation of imprinted genes by phthalates has been proposed to generate multigenerational effects [148]. Exposure of newborn mice to phthalates decreased the primordial follicles at puberty and the adults [149]. The exposure during adults results in increased pro-apoptotic gene expression and thus decreased number of primordial or antral follicles [150]. Besides, DBP was found to affect the steroidogenic capacity in human ovaries by the activation of aromatase promotor II and up-regulation of follicle-stimulating hormone receptor (FSHR) in a dose-dependent manner and reach a more robust effect at relative low-concentration near human concentration [151].

15.4.7.4 Leiomyoma

A recent meta-analysis suggested that MECPP exposure was associated with leiomyoma [152]. A study in Taiwan showed that this association may be further increased with the GSTM1 null genotype, and MEP was also found to be significantly associated with leiomyoma [142]. Similar findings were established in the Shanghai for both phthalates [153] and in Korea for MEHP [154] using a case–control design. However, these significant outcomes were not observed in studies using NHANES data [155] or that conducted in Utah [156].

The experiments using human leiomyoma cells demonstrated that DEHP promoted cell viability and anti-apoptotic protein expression, and induced hypoxia inducible factor 1α (HIF-1α) and cyclooxygenase-2 expression [157].

15.4.7.5 Pregnancy Loss

Urinary MEHP levels measured around conception were associated with early pregnancy loss (<20 weeks) using an established cohort of couples planning their first pregnancy in Demark [158]. However, similar measurements were associated with *reduced* early pregnancy loss (≤42 days) for those who discontinued contraception in North Carolina, USA [158]. The difference might be attributable to the different definitions of the time of "early" pregnancy loss. The case–control studies revealed that subjects with recurrent early pregnancy loss had higher levels of urinary MBP in Taiwan [159], and MEHP in China [160] than those without pregnancy loss did. A recent prospective study of pregnant women showed that missed abortion was associated with increased levels of DEHP in the hair [161]. Few mechanistic studies are available. DBP was

found to have a role in multigenerational and fetotoxic effects [162]; a considerable decrease in the weight of placenta, and pre- and post-implantation loss were observed in F1, F2, and F3 generations in DBP-toxicated (F1) rats crossed and reared up to three generations (F2 and F3). Further, there was a decrease in the number of live births and fetal body weight with high mortality. The developmental indices showed the levels of the hormones important for the fetal development such as thyroid profile that may play a certain role [158, 162]. More investigations are needed.

15.4.7.6 Breast Cancer (BCA)

The association between breast cancer (BCA) and phthalate exposure is principally inconclusive, mainly because of the lack of pre-cancerous measurement of the doses (Table 15.3). The case–control study in Mexico found that MEP was significantly associated with BCA [163] and this persisted after anthocyanins and flavan-3-ols were considered. On the other hand, these flavonoids synergistically increased the negative association between BBzP and BCA [164]. In Alaska where PCB, PBDE, and DDT were also studied, only MEHP showed a significant association with BCA (odds ratio [OR] = 2.43 for those with exposure greater than the median level), regardless of an estrogen receptor [ER]- or progesterone receptor [PR]-positive status [165].

In the Long Island BCA study, phthalates were generally negatively associated with BCA [166]. However, obesity might have affected the results, MECPP (a DEHP tertiary metabolite) was associated with poor survival in lean patients (BMI ≤ 25 kg/m^2, hazard ratio [HR] = 2.39 for third vs. first exposure groups), but better in patients who are obese (HR = 0.71, $p < 0.05$). MEP was found to be associated with increased breast density at Tanner stage 4 in pubertal developing girls, who might be a vulnerable population [167]. Finally, paternal exposure to *DBP* was found to be associated with female offspring' BCA risk in New Zealand [168]. The exposure to DEHP or MEHP increased cell proliferation by activating progesterone receptor signaling, which could potentially increase the risks to develop breast cancer in the human breast ductal carcinoma T-47D cells [169].

For further casual inference, prospective cohort studies are particularly needed because phthalates exposure is associated with therapeutic procedures and easily altered through lifestyle changes. In addition, mechanistic investigations considering obesity, protective agent intake [170], genetic factors (i.e., PPARγ and, cytochrome P450 1 [CYP1]), *progesterone receptor signaling* [169], paternal exposure, or co-exposure to other EDCs [171], with sufficient sample size are warranted for future prevention or intervention in BCA.

15.4.7.7 Summary

In summary, the prevalently used phthalates, particularly for the high-molecular-weight ones, were found to be associated with increased risk for various reproductive dysfunctions including leiomyoma, endometroisis, ovarian dysfunction, and pregnancy loss in women and in men with decreased semen quality. BCA promotion and progression were hypothesized to be positively associated with phthalate exposure. Fecundity appeared to be inconclusive also and warrants further investigations in human reproduction. *Prenatal* exposure to endocrine disruptors has been found to be associated with various gynecological diseases including breast cancer, eclampsia, and infertility [135] with epigenetic changes hypothesized [172, 173], which deserve evaluations for the phthalate investigation.

15.5 Conclusions

S. L. Wang

Phthalates exposure was found to be associated with various adverse effects related to altered functions of systems including the endocrine, immune, nervous, and reproduction, particularly at critical development windows during fetal, fast growing, and pubertal stages. BCA promotion and progression were hypothesized to be positively associated with phthalate exposure. Future studies are necessary for cancers of the breast, endometrial tissue, ovary, and/or prostate to understand the potential risk related to sex hormone sensitive neoplasms. Moreover, fecundity is also inconclusive and warrants further investigations in human reproduction. *Prenatal* exposure *to phthalate deserves investigations as well* [174]. Future research focus should be directed to multiple generation approaches (i.e., exposure signature) considering psychological parameters (i.e., stress) to provide a wider observational window for the conclusions.

References

1. Wittassek M, Koch HM, Angerer J, Bruning T. Assessing exposure to phthalates – the human biomonitoring approach. Mol Nutr Food Res. 2011;55:7–31.
2. Wormuth M, Scheringer M, Vollenweider M, Hungerbuhler K. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans? Risk Anal. 2006;26:803–24.
3. Carlstedt F, Jonsson BA, Bornehag CG. PVC flooring is related to human uptake of phthalates in infants. Indoor Air. 2013;23:32–9.
4. Koch HM, Calafat AM. Human body burdens of chemicals used in plastic manufacture. Philos Trans R Soc Lond Ser B Biol Sci. 2009;364:2063–78.

5. Anderson WA, Castle L, Scotter MJ, Massey RC, Springall C. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Addit Contam. 2001;18:1068–74.
6. Koch HM, Angerer J. Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. Int J Hyg Environ Health. 2007;210:9–19.
7. Koch HM, Bolt HM, Angerer J. Di(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP. Arch Toxicol. 2004;78:123–30.
8. Koch HM, Bolt HM, Preuss R, Angerer J. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch Toxicol. 2005;79:367–76.
9. Silva MJ, Reidy JA, Kato K, et al. Assessment of human exposure to di-isodecyl phthalate using oxidative metabolites as biomarkers. Biomarkers. 2007;12:133–44.
10. Wittassek M, Angerer J. Phthalates: metabolism and exposure. Int J Androl. 2008;31:131–8.
11. Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure an update and latest results. Int J Androl. 2006;29:155–65; discussion 181-5
12. Guo Y, Kannan K. Comparative assessment of human exposure to phthalate esters from house dust in China and the United States. Environ Sci Technol. 2011;45:3788–94.
13. Guo Y, Alomirah H, Cho HS, et al. Occurrence of phthalate metabolites in human urine from several Asian countries. Environ Sci Technol. 2011;45:3138–44.
14. Huang PC, Tsai CH, Liang WY, et al. Age and gender differences in urinary levels of eleven phthalate metabolites in general Taiwanese population after a DEHP episode. PLoS One. 2015;10:e0133782.
15. Wen HJ, Chen CC, Wu MT, et al. Phthalate exposure and reproductive hormones and sex-hormone binding globulin before puberty – phthalate contaminated-foodstuff episode in Taiwan. PLoS One. 2017;12:e0175536.
16. Heudorf U, Mersch-Sundermann V, Angerer J. Phthalates: toxicology and exposure. Int J Hyg Environ Health. 2007;210:623–34.
17. Lin S, Ku HY, Su PH, et al. Phthalate exposure in pregnant women and their children in Central Taiwan. Chemosphere. 2011;82:947–55.
18. Hartmann C, Uhl M, Weiss S, et al. Human biomonitoring of phthalate exposure in Austrian children and adults and cumulative risk assessment. Int J Hyg Environ Health. 2015;218:489–99.
19. Ait Bamai Y, Araki A, Kawai T, et al. Comparisons of urinary phthalate metabolites and daily phthalate intakes among Japanese families. Int J Hyg Environ Health. 2015;218:461–70.
20. Li JH, Ko YC. Plasticizer incident and its health effects in Taiwan. Kaohsiung J Med Sci. 2012;28:S17–21.
21. Wu CF, Chen BH, Shiea J, et al. Temporal changes of urinary oxidative metabolites of di(2-ethylhexyl)phthalate after the 2011 phthalate incident in Taiwanese children: findings of a six month follow-up. Environ Sci Technol. 2013;47:13754–62.
22. Huang HB, Chuang CJ, Su PH, et al. Prenatal and childhood exposure to phthalate diesters and thyroid function in a 9-year follow-up birth Cohort study: Taiwan Maternal and Infant Cohort study. Epidemiology. 2017;28(Suppl 1):S10–8.
23. Lien YJ, Ku HY, Su PH, et al. Prenatal exposure to phthalate esters and behavioral syndromes in children at 8 years of age: Taiwan Maternal and Infant Cohort Study. Environ Health Perspect. 2015;123:95–100.
24. Wang IJ, Lin CC, Lin YJ, Hsieh WS, Chen PC. Early life phthalate exposure and atopic disorders in children: a prospective birth cohort study. Environ Int. 2014;62:48–54.
25. Chen CC, Wang YH, Chen WJ, et al. A benchmark dose study of prenatal exposure to di(2-ethylhexyl) phthalate and behavioral problems in children. Int J Hyg Environ Health. 2019;222:971–80.
26. Chen SY, Hwang JS, Sung FC, et al. Mono-2-ethylhexyl phthalate associated with insulin resistance and lower testosterone levels in a young population. Environ Pollut. 2017;225:112–7.

27. Wen HJ, Sie L, Su PH, et al. Prenatal and childhood exposure to phthalate diesters and sex steroid hormones in 2-, 5-, 8-, and 11-year-old children: a pilot study of the Taiwan Maternal and Infant Cohort Study. J Epidemiol. 2017;27:516–23.
28. Lin LC, Wang SL, Chang YC, et al. Associations between maternal phthalate exposure and cord sex hormones in human infants. Chemosphere. 2011;83:1192–9.
29. Araki A, Mitsui T, Miyashita C, et al. Association between maternal exposure to di(2-ethylhexyl) phthalate and reproductive hormone levels in fetal blood: the Hokkaido study on environment and children's health. PLoS One. 2014;9:e109039.
30. Sathyanarayana S, Butts S, Wang C, et al. Early prenatal phthalate exposure, sex steroid hormones, and birth outcomes. J Clin Endocrinol Metab. 2017;102:1870–8.
31. Fong JP, Lee FJ, Lu IS, Uang SN, Lee CC. Relationship between urinary concentrations of di(2-ethylhexyl) phthalate (DEHP) metabolites and reproductive hormones in polyvinyl chloride production workers. Occup Environ Med. 2015;72:346–53.
32. Swan SH, Main KM, Liu F, et al. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect. 2005;113:1056–61.
33. Sathyanarayana S, Grady R, Barrett ES, et al. First trimester phthalate exposure and male newborn genital anomalies. Environ Res. 2016;151:777–82.
34. Martino-Andrade AJ, Liu F, Sathyanarayana S, et al. Timing of prenatal phthalate exposure in relation to genital endpoints in male newborns. Andrology. 2016;4:585–93.
35. Swan SH, Sathyanarayana S, Barrett ES, et al. First trimester phthalate exposure and anogenital distance in newborns. Hum Reprod. 2015;30:963–72.
36. Wenzel AG, Bloom MS, Butts CD, et al. Influence of race on prenatal phthalate exposure and anogenital measurements among boys and girls. Environ Int. 2018;110:61–70.
37. Bornehag CG, Carlstedt F, Jonsson BA, et al. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ Health Perspect. 2015;123:101–7.
38. Axelsson J, Rylander L, Rignell-Hydbom A, et al. Prenatal phthalate exposure and reproductive function in young men. Environ Res. 2015;138:264–70.
39. Su PH, Chang CK, Lin CY, et al. Prenatal exposure to phthalate ester and pubertal development in a birth cohort in Central Taiwan: a 12-year follow-up study. Environ Res. 2015;136:324–30.
40. Watkins DJ, Sanchez BN, Tellez-Rojo MM, et al. Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ Res. 2017;159:143–51.
41. Watkins DJ, Sanchez BN, Tellez-Rojo MM, et al. Impact of phthalate and BPA exposure during in utero windows of susceptibility on reproductive hormones and sexual maturation in peripubertal males. Environ Health. 2017;16:69.
42. Kasper-Sonnenberg M, Wittsiepe J, Wald K, Koch HM, Wilhelm M. Pre-pubertal exposure with phthalates and bisphenol A and pubertal development. PLoS One. 2017;12:e0187922.
43. Borch J, Metzdorff SB, Vinggaard AM, Brokken L, Dalgaard M. Mechanisms underlying the anti-androgenic effects of diethylhexyl phthalate in fetal rat testis. Toxicology. 2006;223:144–55.
44. Hallmark N, Walker M, McKinnell C, et al. Effects of monobutyl and di(n-butyl) phthalate in vitro on steroidogenesis and Leydig cell aggregation in fetal testis explants from the rat: comparison with effects in vivo in the fetal rat and neonatal marmoset and in vitro in the human. Environ Health Perspect. 2007;115:390–6.
45. Kim DH, Park CG, Kim SH, Kim YJ. The effects of mono-(2-Ethylhexyl) phthalate (MEHP) on human estrogen receptor (hER) and androgen receptor (hAR) by YES/YAS in vitro assay. Molecules. 2019;24:E1558.
46. Kuo FC, Su SW, Wu CF, et al. Relationship of urinary phthalate metabolites with serum thyroid hormones in pregnant women and their newborns: a prospective birth cohort in Taiwan. PLoS One. 2015;10:e0123884.
47. Wu MT, Wu CF, Chen BH, et al. Intake of phthalate-tainted foods alters thyroid functions in Taiwanese children. PLoS One. 2013;8:e55005.

48. Morgenstern R, Whyatt RM, Insel BJ, et al. Phthalates and thyroid function in preschool age children: sex specific associations. Environ Int. 2017;106:11–8.
49. de Cock M, de Boer MR, Lamoree M, Legler J, van de Bor M. Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants – a Dutch prospective cohort study. Environ Health. 2014;13:106.
50. Yao HY, Han Y, Gao H, et al. Maternal phthalate exposure during the first trimester and serum thyroid hormones in pregnant women and their newborns. Chemosphere. 2016;157:42–8.
51. Boas M, Frederiksen H, Feldt-Rasmussen U, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 2010;118:1458–64.
52. Meeker JD, Calafat AM, Hauser R. Di(2-ethylhexyl) phthalate metabolites may alter thyroid hormone levels in men. Environ Health Perspect. 2007;115:1029–34.
53. Meeker JD, Ferguson KK. Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007-2008. Environ Health Perspect. 2011;119:1396–402.
54. Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum Reprod. 2007;22:2715–22.
55. Johns LE, Ferguson KK, McElrath TF, Mukherjee B, Meeker JD. Associations between repeated measures of maternal urinary phthalate metabolites and thyroid hormone parameters during pregnancy. Environ Health Perspect. 2016;124:1808–15.
56. Schug TT, Janesick A, Blumberg B, Heindel JJ. Endocrine disrupting chemicals and disease susceptibility. J Steroid Biochem Mol Biol. 2011;127:204–15.
57. Howarth JA, Price SC, Dobrota M, Kentish PA, Hinton RH. Effects on male rats of di-(2-ethylhexyl) phthalate and di-n-hexylphthalate administered alone or in combination. Toxicol Lett. 2001;121:35–43.
58. Poon R, Lecavalier P, Mueller R, et al. Subchronic oral toxicity of di-n-octyl phthalate and di(2-Ethylhexyl) phthalate in the rat. Food Chem Toxicol. 1997;35:225–39.
59. Shen O, Du G, Sun H, et al. Comparison of in vitro hormone activities of selected phthalates using reporter gene assays. Toxicol Lett. 2009;191:9–14.
60. Shi W, Wang X, Hu G, et al. Bioanalytical and instrumental analysis of thyroid hormone disrupting compounds in water sources along the Yangtze River. Environ Pollut. 2011;159:441–8.
61. Wenzel A, Franz C, Breous E, Loos U. Modulation of iodide uptake by dialkyl phthalate plasticisers in FRTL-5 rat thyroid follicular cells. Mol Cell Endocrinol. 2005;244:63–71.
62. Liu C, Zhao L, Wei L, Li L. DEHP reduces thyroid hormones via interacting with hormone synthesis-related proteins, deiodinases, transthyretin, receptors, and hepatic enzymes in rats. Environ Sci Pollut Res Int. 2015;22:12711–9.
63. Ye H, Ha M, Yang M, et al. Di2-ethylhexyl phthalate disrupts thyroid hormone homeostasis through activating the Ras/Akt/TRHr pathway and inducing hepatic enzymes. Sci Rep. 2017;7:40153.
64. Dong J, Cong Z, You M, et al. Effects of perinatal di (2-ethylhexyl) phthalate exposure on thyroid function in rat offspring. Environ Toxicol Pharmacol. 2019;67:53–60.
65. Rice D, Barone SJ. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect. 2000;108(Suppl 3):511–33.
66. Stroustrup A, Swan SH. In: Landrigan PJ, Etzel RA, editors. Textbook of children's environmental health. New York: Oxford University Press; 2014. Endocrine disruptors. p. 328–9.
67. Kim Y, Ha EH, Kim EJ, et al. Prenatal exposure to phthalates and infant development at 6 months: prospective mothers and Children's environmental health (MOCEH) study. Environ Health Perspect. 2011;119:1495–500.
68. Whyatt RM, Liu X, Rauh VA, et al. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ Health Perspect. 2012;120:290–5.

69. Doherty BT, Engel SM, Buckley JP, et al. Prenatal phthalate biomarker concentrations and performance on the Bayley scales of infant development-II in a population of young urban children. Environ Res. 2017;152:51–8.
70. Tellez-Rojo MM, Cantoral A, Cantonwine DE, et al. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. Sci Total Environ. 2013;461–462:386–90.
71. Factor-Litvak P, Insel B, Calafat AM, et al. Persistent associations between maternal prenatal exposure to phthalates on child IQ at age 7 years. PLoS One. 2014;9:e114003.
72. Huang HB, Chen HY, Su PH, et al. Fetal and childhood exposure to phthalate diesters and cognitive function in children up to 12 years of age: Taiwanese maternal and infant cohort study. PLoS One. 2015;10:e0131910.
73. Kim JI, Hong Y-C, Shin CH, et al. The effects of maternal and children phthalate exposure on the neurocognitive function of 6-year-old children. Environ Res. 2017;156:519–25.
74. Minatoya M, Naka jima S, Sasaki S, et al. Effects of prenatal phthalate exposure on thyroid hormone levels, mental and psychomotor development of infants: the Hokkaido study on environment and Children's health. Sci Total Environ. 2016;565:1037–43.
75. Engel SM, Miodovnik A, Canfield RL, et al. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ Health Perspect. 2010;118:565–71.
76. Kobrosly RW, Evans S, Miodovnik A, et al. Prenatal phthalate exposures and neurobehavioral development scores in boys and girls at 6–10 years of age. Environ Health Perspect. 2014;122:521–8.
77. Chen T, Yang W, Li Y, Chen X, Xu S. Mono-(2-ethylhexyl) phthalate impairs neurodevelopment: inhibition of proliferation and promotion of differentiation in PC12 cells. Toxicol Lett. 2011;201:34–41.
78. Dhanya CR, Indu AR, Deepadevi KV, Kurup PA. Inhibition of membrane Na(+)-K+ Atpase of the brain, liver and RBC in rats administered di(2-ethyl hexyl) phthalate (DEHP) a plasticizer used in polyvinyl chloride (PVC) blood storage bags. Indian J Exp Biol. 2003;41:814–20.
79. Wang R, Xu X, Zhu Q. Pubertal exposure to di-(2-ethylhexyl) phthalate influences social behavior and dopamine receptor D2 of adult female mice. Chemosphere. 2016;144:1771–9.
80. Kota BP, Huang TH, Roufogalis BD. An overview on biological mechanisms of PPARs. Pharmacol Res. 2005;51:85–94.
81. van Neerven S, Kampmann E, Mey J. RAR/RXR and PPAR/RXR signaling in neurological and psychiatric diseases. Prog Neurobiol. 2008;85:433–51.
82. Akaike M, Kato N, Ohno H, Kobayashi T. Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. Neurotoxicol Teratol. 1991;13:317–22.
83. Stein SA, Adams PM, Shanklin DR, Mihailoff GA, Palnitkar MB. Thyroid hormone control of brain and motor development: molecular, neuroanatomical, and behavioral studies. Adv Exp Med Biol. 1991;299:47–105.
84. Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC phases one and three repeat multicountry cross-sectional surveys. Lancet. 2006;368:733–43.
85. Balkrishnan R, Housman TS, Carroll C, Feldman SR, Fleischer AB. Disease severity and associated family impact in childhood atopic dermatitis. Arch Dis Child. 2003;88:423–7.
86. Li MC, Chen CH, Guo YL. Phthalate esters and childhood asthma: a systematic review and congener-specific meta-analysis. Environ Pollut. 2017;229:655–60.
87. Jaakkola JJ, Knight TL. The role of exposure to phthalates from polyvinyl chloride products in the development of asthma and allergies: a systematic review and meta-analysis. Environ Health Perspect. 2008;116:845–53.
88. Ait Bamai Y, Shibata E, Saito I, et al. Exposure to house dust phthalates in relation to asthma and allergies in both children and adults. Sci Total Environ. 2014;485-486:153–63.

89. Deutschle T, Reiter R, Butte W, et al. A controlled challenge study on di(2-ethylhexyl) phthalate (DEHP) in house dust and the immune response in human nasal mucosa of allergic subjects. Environ Health Perspect. 2008;116:1487–93.
90. Bertelsen RJ, Carlsen KC, Calafat AM, et al. Urinary biomarkers for phthalates associated with asthma in Norwegian children. Environ Health Perspect. 2013;121:251–6.
91. Ku HY, Su PH, Wen HJ, et al. Prenatal and postnatal exposure to phthalate esters and asthma: a 9-year follow-up study of a Taiwanese birth cohort. PLoS One. 2015;10:e0123309.
92. Ait Bamai Y, Miyashita C, Araki A, et al. Effects of prenatal di(2-ethylhexyl) phthalate exposure on childhood allergies and infectious diseases: the Hokkaido study on environment and Children's health. Sci Total Environ. 2018;618:1408–15.
93. Jahreis S, Trump S, Bauer M, et al. Maternal phthalate exposure promotes allergic airway inflammation over 2 generations through epigenetic modifications. J Allergy Clin Immunol. 2018;141:741–53.
94. Gascon M, Casas M, Morales E, et al. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. J Allergy Clin Immunol. 2015; 135:370–8.
95. Herberth G, Pierzchalski A, Feltens R, et al. Prenatal phthalate exposure associates with low regulatory T-cell numbers and atopic dermatitis in early childhood: results from the LINA mother-child study. J Allergy Clin Immunol. 2017;139:1376–1379 e8.
96. Choi WJ, Kwon HJ, Hong S, et al. Potential nonmonotonous association between di(2-ethylhexyl) phthalate exposure and atopic dermatitis in Korean children. Br J Dermatol. 2014;171:854–60.
97. Overgaard LEK, Main KM, Frederiksen H, et al. Children with atopic dermatitis and frequent emollient use have increased urinary levels of low-molecular-weight phthalate metabolites and parabens. Allergy. 2017;72:1768–77.
98. Wang IJ, Karmaus WJ. The effect of phthalate exposure and filaggrin gene variants on atopic dermatitis. Environ Res. 2015;136:213–8.
99. Guo J, Han B, Qin L, et al. Pulmonary toxicity and adjuvant effect of di-(2-exylhexyl) phthalate in ovalbumin-immunized BALB/c mice. PLoS One. 2012;7:e39008.
100. Kuo CH, Hsieh CC, Kuo HF, et al. Phthalates suppress type I interferon in human plasmacytoid dendritic cells via epigenetic regulation. Allergy. 2013;68:870–9.
101. Han Y, Wang X, Chen G, et al. Di-(2-ethylhexyl) phthalate adjuvantly induces imbalanced humoral immunity in ovalbumin-sensitized BALB/c mice ascribing to T follicular helper cells hyperfunction. Toxicology. 2014;324:88–97.
102. Pei X, Duan Z, Ma M, Zhang Y, Guo L. Role of Ca/CaN/NFAT signaling in IL-4 expression by splenic lymphocytes exposed to phthalate (2-ethylhexyl) ester in spleen lymphocytes. Mol Biol Rep. 2014;41:2129–42.
103. You H, Li R, Wei C, et al. Thymic stromal Lymphopoietin neutralization inhibits the immune adjuvant effect of Di-(2-Ethylhexyl) phthalate in Balb/c mouse asthma model. PLoS One. 2016;11:e0159479.
104. Shen S, Li J, You H, et al. Oral exposure to diisodecyl phthalate aggravates allergic dermatitis by oxidative stress and enhancement of thymic stromal lymphopoietin. Food Chem Toxicol. 2017;99:60–9.
105. Bolling AK, Ovrevik J, Samuelsen JT, et al. Mono-2-ethylhexylphthalate (MEHP) induces TNF-alpha release and macrophage differentiation through different signalling pathways in RAW264.7 cells. Toxicol Lett. 2012;209:43–50.
106. World Health Organisation (WHO). Obesity and overweight. http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight. Accessed 16 February 2018.
107. Minatoya M, Araki A, Miyashita C, et al. Association between prenatal bisphenol A and phthalate exposures and fetal metabolic related biomarkers: the Hokkaido study on environment and Children's health. Environ Res. 2018;161:505–11.
108. Buckley JP, Engel SM, Braun JM, et al. Prenatal phthalate exposures and body mass index among 4- to 7-year-old children: a pooled analysis. Epidemiology. 2016;27:449–58.

109. Hatch EE, Nelson JW, Qureshi MM, et al. Association of urinary phthalate metabolite concentrations with body mass index and waist circumference: a cross-sectional study of NHANES data, 1999–2002. Environ Health. 2008;7:27.
110. Wang H, Zhou Y, Tang C, et al. Urinary phthalate metabolites are associated with body mass index and waist circumference in Chinese school children. PLoS One. 2013;8:e56800.
111. Hou JW, Lin CL, Tsai YA, et al. The effects of phthalate and nonylphenol exposure on body size and secondary sexual characteristics during puberty. Int J Hyg Environ Health. 2015;218:603–15.
112. Trasande L, Attina TM, Sathyanarayana S, Spanier AJ, Blustein J. Race/ethnicity-specific associations of urinary phthalates with childhood body mass in a nationally representative sample. Environ Health Perspect. 2013;121:501–6.
113. Maresca MM, Hoepner LA, Hassoun A, et al. Prenatal exposure to phthalates and childhood body size in an urban cohort. Environ Health Perspect. 2016;124:514–20.
114. Grun F, Blumberg B. Environmental obesogens: organotins and endocrine disruption via nuclear receptor signaling. Endocrinology. 2006;147:S50–5.
115. Taxvig C, Dreisig K, Boberg J, et al. Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARgamma activation. Mol Cell Endocrinol. 2012;361:106–15.
116. Sonkar R, Powell CA, Choudhury M. Benzyl butyl phthalate induces epigenetic stress to enhance adipogenesis in mesenchymal stem cells. Mol Cell Endocrinol. 2016;431:109–22.
117. Schmidt JS, Schaedlich K, Fiandanese N, Pocar P, Fischer B. Effects of di(2-ethylhexyl) phthalate (DEHP) on female fertility and adipogenesis in C3H/N mice. Environ Health Perspect. 2012;120:1123–9.
118. Kloting N, Hesselbarth N, Gericke M, et al. Di-(2-Ethylhexyl)-phthalate (DEHP) causes impaired adipocyte function and alters serum metabolites. PLoS One. 2015;10:e0143190.
119. Rais-Bahrami K, Nunez S, Revenis ME, Luban NL, Short BL. Follow-up study of adolescents exposed to di(2-ethylhexyl) phthalate (DEHP) as neonates on extracorporeal membrane oxygenation (ECMO) support. Environ Health Perspect. 2004;112:1339–40.
120. Trasande L, Sathyanarayana S, Trachtman H. Dietary phthalates and low-grade albuminuria in US children and adolescents. Clin J Am Soc Nephrol. 2014;9:100–9.
121. Tsai HJ, Chen BH, Wu CF, et al. Intake of phthalate-tainted foods and microalbuminuria in children: the 2011 Taiwan food scandal. Environ Int. 2016;89-90:129–37.
122. Marsman D. NTP technical report on the toxicity studies of Dibutyl phthalate (CAS no. 84-74-2) administered in feed to F344/N rats and B6C3F1 mice. Toxic Rep Ser. 1995;30:1–G5.
123. Pugh G Jr, Isenberg JS, Kamendulis LM, et al. Effects of di-isononyl phthalate, di-2-ethylhexyl phthalate, and clofibrate in cynomolgus monkeys. Toxicol Sci. 2000;56:181–8.
124. David RM, Moore MR, Finney DC, Guest D. Chronic toxicity of di(2-ethylhexyl)phthalate in mice. Toxicol Sci. 2000;58:377–85.
125. Kamijo Y, Hora K, Nakajima T, et al. Peroxisome proliferator-activated receptor alpha protects against glomerulonephritis induced by long-term exposure to the plasticizer di-(2-ethylhexyl)phthalate. J Am Soc Nephrol. 2007;18:176–88.
126. Zhu YP, Chen L, Wang XJ, et al. Maternal exposure to di-n-butyl phthalate (DBP) induces renal fibrosis in adult rat offspring. Oncotarget. 2017;8:31101–11.
127. Wu CT, Wang CC, Huang LC, Liu SH, Chiang CK. Plasticizer di-(2-Ethylhexyl)phthalate induces epithelial-to-mesenchymal transition and renal fibrosis in vitro and in vivo. Toxicol Sci. 2018;164:363–74.
128. Bloom MS, Whitcomb BW, Chen Z, et al. Associations between urinary phthalate concentrations and semen quality parameters in a general population. Hum Reprod. 2015;30:2645–57.
129. Thurston SW, Mendiola J, Bellamy AR, et al. Phthalate exposure and semen quality in fertile US men. Andrology. 2016;4:632–8.
130. Chang WH, Wu MH, Pan HA, Guo PL, Lee CC. Semen quality and insulin-like factor 3: associations with urinary and seminal levels of phthalate metabolites in adult males. Chemosphere. 2017;173:594–602.

131. Pan Y, Jing J, Yeung LWY, et al. Associations of urinary 5-methyl-2′-deoxycytidine and 5-hydroxymethyl-2′-deoxycytidine with phthalate exposure and semen quality in 562 Chinese adult men. Environ Int. 2016;94:583–90.
132. Al-Saleh I, Coskun S, Al-Doush I, et al. The relationships between urinary phthalate metabolites, reproductive hormones and semen parameters in men attending in vitro fertilization clinic. Sci Total Environ. 2019;658:982–95.
133. Wang YX, You L, Zeng Q, et al. Phthalate exposure and human semen quality: results from an infertility clinic in China. Environ Res. 2015;142:1–9.
134. Ivell R, Heng K, Anand-Ivell R. Insulin-like factor 3 and the HPG axis in the male. Front Endocrinol (Lausanne). 2014;5:6.
135. Aly HA, Hassan MH, El-Beshbishy HA, Alahdal AM, Osman AM. Dibutyl phthalate induces oxidative stress and impairs spermatogenesis in adult rats. Toxicol Ind Health. 2016;32:1467–77.
136. Buck Louis GM, Sundaram R, Sweeney AM, et al. Urinary bisphenol A, phthalates, and couple fecundity: the longitudinal investigation of fertility and the environment (LIFE) study. Fertil Steril. 2014;101:1359–66.
137. Thomsen AM, Riis AH, Olsen J, et al. Female exposure to phthalates and time to pregnancy: a first pregnancy planner study. Hum Reprod. 2017;32:232–8.
138. Minguez-Alarcon L, Gaskins AJ. Female exposure to endocrine disrupting chemicals and fecundity: a review. Curr Opin Obstet Gynecol. 2017;29:202–11.
139. Reddy BS, Rozati R, Reddy BV, Raman NV. Association of phthalate esters with endometriosis in Indian women. BJOG. 2006;113:515–20.
140. Kim SH, Chun S, Jang JY, et al. Increased plasma levels of phthalate esters in women with advanced-stage endometriosis: a prospective case-control study. Fertil Steril. 2011;95:357–9.
141. Kim SH, Cho S, Ihm HJ, et al. Possible role of phthalate in the pathogenesis of endometriosis: in vitro, animal, and human data. J Clin Endocrinol Metab. 2015;100:E1502–11.
142. Huang PC, Tsai EM, Li WF, et al. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. Hum Reprod. 2010;25:986–94.
143. Messerlian C, Souter I, Gaskins AJ, et al. Urinary phthalate metabolites and ovarian reserve among women seeking infertility care. Hum Reprod. 2016;31:75–83.
144. Vagi SJ, Azziz-Baumgartner E, Sjodin A, et al. Exploring the potential association between brominated diphenyl ethers, polychlorinated biphenyls, organochlorine pesticides, perfluorinated compounds, phthalates, and bisphenol A in polycystic ovary syndrome: a case-control study. BMC Endocr Disord. 2014;14:86.
145. Cho YJ, Park SB, Han M. Di-(2-ethylhexyl)-phthalate induces oxidative stress in human endometrial stromal cells in vitro. Mol Cell Endocrinol. 2015;407:9–17.
146. Kim Y, Kim MR, Kim JH, Cho HH. Aldo-keto reductase activity after diethylhexyl phthalate exposure in eutopic and ectopic endometrial cells. Eur J Obstet Gynecol Reprod Biol. 2017;215:215–9.
147. Moyer B, Hixon ML. Reproductive effects in F1 adult females exposed in utero to moderate to high doses of mono-2-ethylhexylphthalate (MEHP). Reprod Toxicol. 2012;34:43–50.
148. Vabre P, Gatimel N, Moreau J, et al. Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environ Health. 2017;16:37.
149. Zhang XF, Zhang LJ, Li L, et al. Diethylhexyl phthalate exposure impairs follicular development and affects oocyte maturation in the mouse. Environ Mol Mutagen. 2013;54:354–61.
150. Hannon PR, Niermann S, Flaws JA. Acute exposure to Di(2-Ethylhexyl) phthalate in adulthood causes adverse reproductive outcomes later in life and accelerates reproductive aging in female mice. Toxicol Sci. 2016;150:97–108.

151. Ma Y, Zhang J, Zeng R, et al. Effects of the Dibutyl phthalate (DBP) on the expression and activity of aromatase in human Granulosa cell line KGN. Ann Clin Lab Sci. 2019;49:175–82.
152. Fu Z, Zhao F, Chen K, et al. Association between urinary phthalate metabolites and risk of breast cancer and uterine leiomyoma. Reprod Toxicol. 2017;74:134–42.
153. Sun J, Zhang MR, Zhang LQ, et al. Phthalate monoesters in association with uterine leiomyomata in Shanghai. Int J Environ Health Res. 2016;26:306–16.
154. Kim YA, Kho Y, Chun KC, et al. Increased urinary phthalate levels in women with uterine leiomyoma: a case-control study. Int J Environ Res Public Health 2016; 13:E1247.
155. Weuve J, Hauser R, Calafat AM, Missmer SA, Wise LA. Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999–2004. Environ Health Perspect. 2010;118:825–32.
156. Pollack AZ, Buck Louis GM, Chen Z, et al. Bisphenol A, benzophenone-type ultraviolet filters, and phthalates in relation to uterine leiomyoma. Environ Res. 2015;137:101–7.
157. Kim JH. Analysis of the in vitro effects of di-(2-ethylhexyl) phthalate exposure on human uterine leiomyoma cells. Exp Ther Med. 2018;15:4972–8.
158. Jukic AM, Calafat AM, McConnaughey DR, et al. Urinary concentrations of phthalate metabolites and Bisphenol A and associations with follicular-phase length, luteal-phase length, Fecundability, and early pregnancy loss. Environ Health Perspect. 2016; 124:321–8.
159. Liao KW, Kuo PL, Huang HB, et al. Increased risk of phthalates exposure for recurrent pregnancy loss in reproductive-aged women. Environ Pollut. 2018;241:969–77.
160. Peng F, Ji W, Zhu F, et al. A study on phthalate metabolites, bisphenol A and nonylphenol in the urine of Chinese women with unexplained recurrent spontaneous abortion. Environ Res. 2016;150:622–8.
161. Zhao R, Wu Y, Zhao F, et al. The risk of missed abortion associated with the levels of tobacco, heavy metals and phthalate in hair of pregnant woman: a case control study in Chinese women. Medicine (Baltimore). 2017;96:e9388.
162. Mahaboob Basha P, Radha MJ. Gestational di-n-butyl phthalate exposure induced developmental and teratogenic anomalies in rats: a multigenerational assessment. Environ Sci Pollut Res Int. 2017;24:4537–51.
163. Lopez-Carrillo L, Hernandez-Ramirez RU, Calafat AM, et al. Exposure to phthalates and breast cancer risk in northern Mexico. Environ Health Perspect. 2010;118:539–44.
164. Merida-Ortega A, Hernandez-Alcaraz C, Hernandez-Ramirez RU, et al. Phthalate exposure, flavonoid consumption and breast cancer risk among Mexican women. Environ Int. 2016;96:167–72.
165. Holmes AK, Koller KR, Kieszak SM, et al. Case-control study of breast cancer and exposure to synthetic environmental chemicals among Alaska native women. Int J Circumpolar Health. 2014;73:25760.
166. Parada H Jr, Gammon MD, Chen J, et al. Urinary phthalate metabolite concentrations and breast cancer incidence and survival following breast cancer: the Long Island Breast Cancer Study project. Environ Health Perspect. 2018;126:047013.
167. Binder AM, Corvalan C, Pereira A, et al. Pre-pubertal and pubertal endocrine disrupting chemicals exposure and breast density among Chilean adolescents. Cancer Epidemiol Biomark Prev. 2018;27:1491.
168. Carran M, Shaw IC. New Zealand Malayan war veterans' exposure to dibutylphthalate is associated with an increased incidence of cryptorchidism, hypospadias and breast cancer in their children. N Z Med J. 2012;125:52–63.
169. Crobeddu B, Ferraris E, Kolasa E, Plante I. Di(2-ethylhexyl) phthalate (DEHP) increases proliferation of epithelial breast cancer cells through progesterone receptor dysregulation. Environ Res. 2019;173:165–73.

170. Martinez-Nava GA, Burguete-Garcia AI, Lopez-Carrillo L, et al. PPARγ and PPARGC1B polymorphisms modify the association between phthalate metabolites and breast cancer risk. Biomarkers. 2013;18:493–501.
171. Morgan M, Deoraj A, Felty Q, Roy D. Environmental estrogen-like endocrine disrupting chemicals and breast cancer. Mol Cell Endocrinol. 2017;457:89–102.
172. Tindula G, Murphy SK, Grenier C, et al. DNA methylation of imprinted genes in Mexican-American newborn children with prenatal phthalate exposure. Epigenomics. 2018;10:1011–26.
173. Chen CH, Jiang SS, Chang IS, et al. Association between fetal exposure to phthalate endocrine disruptor and genome-wide DNA methylation at birth. Environ Res. 2018;162:261–70.
174. Chen CC, Wang SL, Wu MT, et al. Exposure estimation for risk assessment of the phthalate incident in Taiwan. PLoS One. 2016;11:e0151070.

Chapter 16
Bisphenols and Alkylphenols

Mei-Lien Chen, Chia-Huang Chang, and Machiko Minatoya

Abstract This chapter provides a general introduction to bisphenols and alkylphenols in toxicity, exposure assessment, biomonitoring, and epidemiological studies. Both bisphenols and alkylphenols are applied intensively to the majority of consumer products and are ubiquitous in environmental matrices. Bisphenol A (BPA), nonylphenol (NP), octylphenol (OP), triclosan (TCS), 3-benzophenone (BP-3), 2,4-dichlorophenol (2,4-DCP), and 2,5-dichlorophenol (2,5-DCP) have been proven as *endocrine-disrupting chemicals (EDCs) and* exhibited potential health effects on human, especially for vulnerable pregnant women and children. This chapter discusses human exposure source, pathway, metabolism, and possible effects of bisphenols and alkylphenols and provides various epidemiological evidences for human health risks.

Keywords Bisphenol · Nonylphenol · Exposure assessment · Biomonitoring Health effects

16.1 Introduction

Over the past decades, bisphenols and alkylphenols are the chemicals extensively used in industrial application, especially for plastic manufacturing process. Bisphenols are used majorly in the manufacture of polycarbonate and alkylphenols are used to produce antioxidants and stabilizers in the production of plastics. In addition, alkylphenol ethoxylates are the most widely used nonionic surfactants. The widespread application of these substances leads to the ubiquitous occurrence in the environment. The increasing concerns with respect to

M.-L. Chen (✉) · C.-H. Chang
Institute of Environmental and Occupational Health Sciences, School of Medicine, National Yang Ming University, Taipei, Taiwan
e-mail: mlchen@ym.edu.tw

M. Minatoya
Hokkaido University Center for Environmental and Health Sciences, Sapporo, Japan
e-mail: mminatoya@cehs.hokudai.ac.jp

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_16

environmental and health risks arise due to their potential endocrine-disrupting properties. This chapter introduces bisphenols and alkylphenols individually and focuses on the potential human exposure source, pathway, metabolism, and health effects.

16.2 Bisphenols

16.2.1 Introduction

Bisphenols are a group of chemical compounds with two hydroxyphenyl functionalities characterized by a shared chemical structure that is optimal for substitution (e.g., carbon or sulfur atom; Fig. 16.1). More than 20 bisphenols exist; among them, bisphenols A, S, and F are most commonly used and most highly produced. Bisphenol A (BPA) is one of the most thoroughly studied endocrine disruptors. It has been identified as a reproductive toxicant, obesogen, and thyroid-disrupting chemical, and cause of neurological disorders [1–5]. BPA is a bisphenol synthesized from the reaction of acetone (A) with phenol. BPA is mainly used to produce polycarbonate (PC) and epoxy resins, and it accounts for 68% and 28% of BPA, respectively [6–8]. PC is used in the manufacture of modern optical media, such as DVDs and CDs, sports equipment, medical devices (dental sealants), food storage containers, reusable bottles (e.g., baby feeding bottles), building and construction materials, automotive parts, and appliances. Epoxy resins are used in internal coatings of food and beverage cans to protect food and drink from direct contact with metals [9, 10]. BPA is also used in ther-

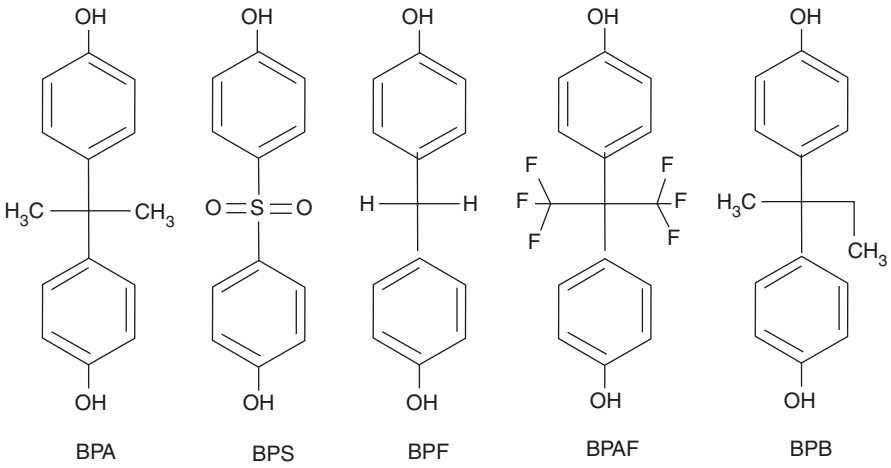


Fig. 16.1 Structure of BPA and its analogs

mal papers, such as credit card slips, bank receipts, and fax papers [11]. BPA may be released into the environment during the manufacture, transport, application, disposal, and incineration of the numerous consumer products in which it is used; consequently, BPA is detectable in ambient air, wastewater, surface water, and sediment [12–16]. Numerous studies have identified BPA in urban ecosystems of Asia, Europe, and North America. Reported concentrations of BPA have ranged from undetectable to 370 mg/L in wastewater treatment plants, undetectable to 56 mg/L in surface water, 10 to more than 100,000 mg/kg dry weight in sludge, and 0.2–13,000 ng/g in wildlife [17]. Dietary intake accounts for more than 90% of overall BPA exposure for toddlers, children, and adults [18]. BPA has been detected in most canned foods, dairy products, and infant formulas, as well as in rice, poultry, livestock, seafood, fruits, and vegetables [19]. Ingested BPA is rapidly absorbed in the gastrointestinal tract, after which the main glucuronic acid-conjugated metabolite forms in the liver. The BPA metabolite is then excreted in urine; the half-life of this metabolite is approximately 6 h [20]. A recent study indicated that the half-life of BPA is considerably longer than anticipated, which suggests that BPA may accumulate in body tissues [21]. Given the ubiquity of BPA in the environment, human exposure to BPA is virtually universal. More than 90% of participants in the National Health and Nutrition Examination Survey (NHANES) had detectable levels of BPA in their urine [22]. Due to the ubiquitous presence of BPA in low concentrations as well as its toxicological effects in vitro and in vivo, concerns regarding the influence of BPA on human health are increasing [23–27].

BPA exerts estrogenic and antiandrogenic effects by interacting with estrogen receptors (ERs) and antagonizing the androgen receptor (AR); these activities interfere with the function of the endocrine system and disrupt the normal signal pathways [28, 29]. In animal studies, BPA exposure has uterotrophic effects, reduces spermatogenesis, accelerates puberty, as well as alters postnatal growth and patterns of estrous cyclicity [30–32]. In utero exposure to BPA may increase the risks of pregnancy failure, embryonal death, impaired growth, and low birth weight [33, 34]. In vitro studies demonstrated that the BPA metabolite can affect adipogenesis and adipocyte metabolic functions and act as an obesogen [35, 36]. Owing to these health concerns, manufacturers have begun developing BPA substitutes, such as bisphenol F (BPF) and bisphenol S (BPS) [37, 38]. Similar to BPA, BPS and BPF are detectable in indoor dust, surface water, sediment, and sewage effluent [39–41]. These three bisphenol compounds (in descending order according to environmental concentrations: BPA, BPS, BPF) account for more than 98% of total environmental bisphenol [39–41]. All bisphenol compounds are associated with an ER-dependent mechanism and thus induce estrogenic responses [42]. Initial studies have indicated that BPA analogs may cause toxic effects similar to those of BPA [43, 44]. Based on the reviews of potential BPA exposure and toxicity, the European Food Safety Authorities (EFSA) reduced the temporary tolerable daily intake (TDI) for BPA from 50 to 4 μg/kg body weight (BW)/day in January, 2015.

16.2.2 Bisphenol Metabolism

BPA metabolism in mammals is mainly driven by phase 2 conjugation, involving glucuronidation and sulfation (Fig. 16.2). BPA ingested by animals or humans is rapidly absorbed in the gastrointestinal tract with the glucuronic acid/sulfotransferases-conjugated metabolite forming in the liver and subsequently excreted through urine. The glucuronidation of BPA is mainly catalyzed by hepatic uridine 5′-diphospho-glucuronosyltransferases (UGTs) [45]. Sulfation is a minor metabolic pathway for BPA, involving catalysis by sulfotransferase [46]. One study processed 30 urine samples treated with or without β-glucuronidase to determine the distribution of BPA metabolites in urine. The results indicated that BPA glucuronide was the predominant metabolite (69.5%), followed by BPA sulfate (21.0%) and unconjugated BPA (9.5%) [47]. Another study also determined that BPA was excreted mainly as glucuronide (>90%) in urine [48]. Other minor metabolites, such as glucuronide/sulfate diconjugates, conjugated methoxylated or hydroxylated compounds, oxidative metabolites of BPA (ortho-hydroxylated BPA), and DNA

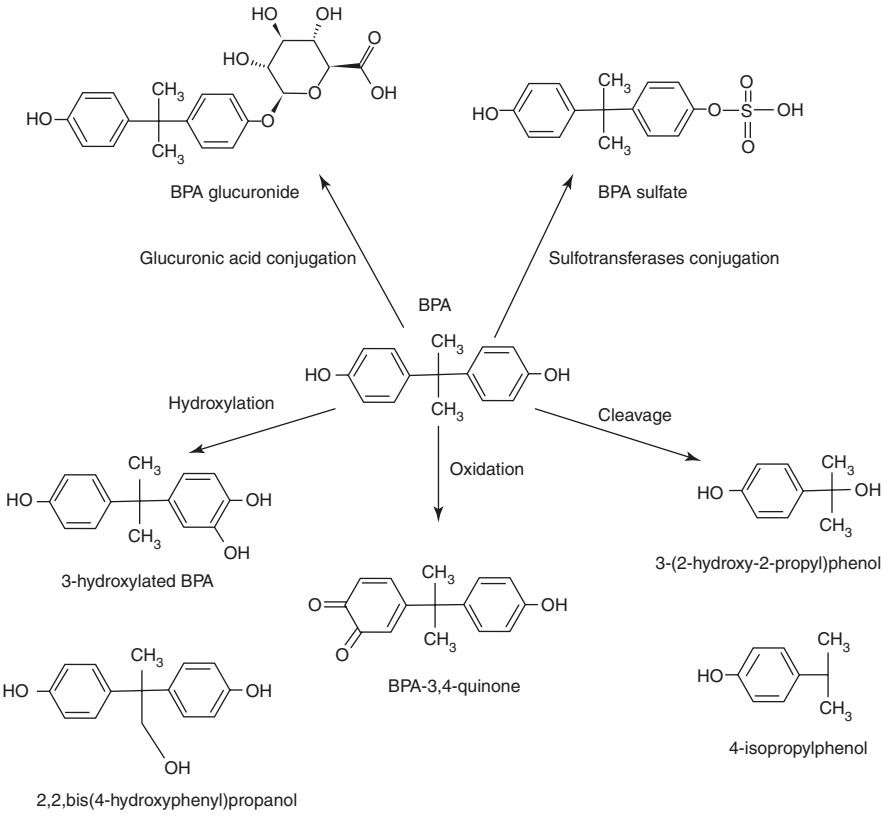


Fig. 16.2 Proposed biotransformation pathways of BPA

adduct (reactive BPA ortho-quinones), have also been identified in vitro and in vivo [49–52]. In one study, three men and three women ingested a low oral dose of BPA. The resultant BPA concentrations in the participants' urine and blood were lower than the detection limit, and the BPA glucuronide concentration could be eliminated from blood through urine in less than 6 h [20]. Furthermore, a few studies have demonstrated that BPA can cross the human placenta, mainly in an active unconjugated form, which was detectable in umbilical cord blood [53, 54]. However, in the fetal liver, the endogenous concentrations of the UGTs that mediate glucuronidation were quite low, indicating that fetuses may be harmed by insufficient detoxification of conjugated BPA [54, 55].

16.2.3 Bisphenol Exposure Assessment

The dominant source of human exposure to BPA is dietary ingestion; food may be contaminated from cans and plastic products. BPA is susceptible to heat, acid, alkali, and alcohol, and thus is easily released from the lacquer surfaces of metal cans and plastic containers and dissolved into foods [9, 10]. BPA can be detected in vegetable cans, beverage cans, meat products, dairy products, and infant formula. The transfer of BPA from a container surface to stored food depends on the temperature and the duration of heat exposure [56]. Kawamura et al. reported that the maximum and average BPA concentrations in domestic canned food in Japan were 30 and 3.4 ng/g, respectively, and the levels in imported canned foods were 390 and 57 ng/g, respectively [57]. A 29.8–110% increase in BPA levels was associated with the migration of BPA from can-coating to a liquid formula for 9 of 21 products stored for 10 months at room temperature [58]. Onn Wong et al. indicated that residual BPA was detected in nearly 70% of baby bottles from 28 brands; the bottles were fabricated from PC in the Singaporean market, and their BPA concentrations ranged from 4.01 to 141 mg/kg [59]. BPA has also been detected in various commonly consumed foods, such as rice, poultry, livestock, seafood, fruits, and vegetables. The estimated BPA intake amounts resulting from chicken, pork and beef, and seafood consumption are 22.65–33.77, 66.35–91.70, and 40.78–54.15 g/day, respectively [19]. Another study estimated that BPA intake from canned foods was 12.4 ng/kg BW/day [60]. An assessment of dietary exposure to BPA revealed that canned food contributed to approximately half of total BPA exposure, followed by animal products (20%) [61]. Infants who ingest infant formula as their main nutrient source may experience continual low-level BPA exposure because BPA migrates into formula at various points of production [62]. According the 2015 EFSA report, infants and toddlers experienced the highest dietary exposure to BPA (0.375 μg/kg BW/day) due to their high consumption of food and beverages per kilogram BW. For adolescents, adults, and elderly people, average dietary exposure was 0.116–0.159 μg/kg of BW/day [63]. The NHANES proposed methods to infer daily intakes of BPA using BPA concentration in urine. According to findings from use of these methods, typical BPA intake was 30–70 ng/kg BW/day [64, 65].

Because BPA is a non-persistent chemical with short half-life in the human body, urine samples are optimal for use in BPA biomonitoring. BPA can also be measured from other matrices, such as saliva, adipose tissue, amniotic fluid, placenta, and breast milk, indicating that BPA can enter various parts of the human body [66–72]. In addition to reports of universal food-based exposure to BPA, reports have indicated a BPA detection rate of over 90% of urine samples from the USA, Canada, Germany, and Asian countries [22, 73–75]. Children exhibited higher BPA concentrations than adolescents and adults [22, 73, 74]. Moreover, among German children, the younger age group (3–5 years) exhibited higher BPA concentrations (geometric mean = 3.55 μg/L) than did the older ones (6–8, 9–11, and 12–14 years) [75]. Vandenberg et al. examined more than 80 published human biomonitoring studies, and their analyses indicated that young children were exposed to high BPA levels [72].

Unconjugated BPA has been detected in cord blood, placenta, and amniotic fluid, suggesting BPA can cross the placenta and enter the fetal compartment [66–68, 76–78] (Table 16.1). BPA exposure during fetal development is a substantial concern; an increasing amount of evidence suggests that BPA irreversibly affects prenatal reproductive development [23]. Placental BPA concentrations (1.0–104.9 ng/g) were higher than maternal (0.3–18.9 ng/mL) and fetal (0.2–9.2 ng/mL) plasma BPA concentrations [77]. Another study also revealed high BPA concentrations in placenta (4.4–273.9 ng/g) [68]. A fetus may be continuously exposed to BPA through fetal–maternal circulation. BPA has been measured in amniotic fluid and cord blood [66, 67, 76, 77]. Ikezuki et al. discovered that BPA levels in amniotic fluid during early pregnancy were eight times higher than those during late pregnancy, possibly because of a lower metabolic clearance in the fetal liver during early pregnancy [76]. Aris and Tan et al. reported that the BPA concentrations of fetal cord blood samples were 0.01–4.60 and 0.05–4.05 ng/mL, respectively (detection rates: 95% and 88%, respectively) [66, 67]. Lee et al. measured BPA concentrations in various bodily fluids and tissues of pregnant women. The matrices are listed as follows in ascending order according to BPA concentrations: placenta (0.53 ng/g), breast milk (0.74 ng/mL), maternal serum (1.56 ng/mL), cord serum (1.71 ng/mL), maternal urine (2.86 ng/mL), and neonatal urine (4.75 ng/mL) [78]. Based on a series of biologically plausible values from peer-reviewed articles, a physiologically based pharmacokinetic (PBPK) model for BPA and its analogs have been developed to enable prediction of BPA absorption, distribution, metabolism, and excretion in humans and facilitate the estimation of the internal dose metrics of bisphenols [87–89].

16.2.4 Effects In Vitro and In Vivo

Endocrine-disrupting chemicals have become a concern because of their potential effects on diverse mechanisms: they have estrogenic and antiandrogenic properties, cause oxidative stress, inhibit the cell cycle, and affect cell differentiation [90, 91].

Table 16.1 BPA levels in various biospecimens

Year	Country	Specimen	*N*	Detection method	LOD	Detection rate (%)	Con. range	GM (median)	Mean ± SD	Reference
2005	USA	Adult urine	394	GC-MS	0.1 μg/L	95	0.22–5.18 μg/g cre.[a]	1.36 (1.32)[b]		Calafat et al. [79]
2008	USA	Urine	2517	HPLC-APCI-MS/MS	0.4 μg/L	93	0.4–149.0 μg/L	2.6 (2.7)		Calafat et al. [22]
2011	Spain	Maternal urine	120	LC-MS/MS	0.4 ng/mL	91		(2.2)		Casas et al. [80]
2011	Spain	Children urine	30	LC-MS/MS	0.4 ng/ml	97		(4.2)		Casas et al. [80]
2015	Canada	Maternal urine	1238	GC-MS	0.2 μg/L	92	<LOD-297.8 μg/L	1.1 (1.2)		Arbuckle et al. [81]
2015	China	Maternal urine	339	UPLC-MS/MS	0.2 μg/L	89	<LOD-216.26 μg/g cre.	3.90 (3.85)	19.35	Huo et al. [69]
2015	China	Maternal urine	137	GC-MS	0.12 ng/mL	79	<LOD-121.9 μg/g cre.	2.7(4.0)[b]		Liu et al. [70]
2015	Canada	Infant urine	100	GC-MS	0.2 μg/L	40	<LOD-9.4 μg/L	<LOD (0.2)		Arbuckle et al. [81]
2008	Korea	Maternal serum	300	HPLC-FLD	0.625 μg/L	84	<LOD-66.48 μg/L	3.10 (2.73)	9.04 ± 14.03	Lee et al. [82]
2014	Canada	Maternal serum	61	GC-MS	0.01 ng/mL	97	0.01–4.60 ng/mL		1.36 ± 1.18	Aris [66]
2017	India	Maternal plasma	40	GC-MS	0.52 ng/mL	78		5.83 (7.43)		Sudhanshu et al. [83]
2003	Malaysia	Fetal cord plasma	180	GC-MS	0.05 ng/mL	88	0.05–4.05 ng/mL			Tan et al. [67]
2008	Korea	Fetal cord serum	300	HPLC-FLD	0.625 μg/L	40	<LOD-8.86 μg/L	0.65 (<0.625)	1.13 ± 1.43	Lee et al. [82]
2014	Canada	Fetal cord serum	61	GC-MS	0.01 ng/mL	95	0.01–4.60 ng/mL		1.23 ± 1.04	Aris [66]

(continued)

Table 16.1 (continued)

Year	Country	Specimen	*N*	Detection method	LOD	Detection rate (%)	Con. range	GM (median)	Mean ± SD	Reference
2017	India	Amniotic fluid	40	GC-MS	0.52 ng/mL	70		5.87 (7.75)		Sudhanshu et al. [83]
2014	USA	Placenta	200	GC-MS	1 ng/g	100	4.4–273.9 ng/g		103.4 ± 61.8	Troisi et al. [68]
2016	Spain	Placenta	79	GC-MS	0.2 ng/g	58	0.50–12.30 ng/g	(1.2)	2.59	Fernández et al. [84]
2015	Canada	Meconium	54	GC-MS	0.48 ng/g	54	<LOD-3.93 ng/g	0.52 (0.60)		Arbuckle et al. [81]
2006	USA	Breast milk	20	LC-MS/MS	0.28 ng/mL	90	<LOD-7.3 ng/mL	(1.1)	1.9	Ye et al. [85]
2013	Korea	Colostrum	325	LC-MS/MS		71	<LOD-57.3 ng/mL	(7.8)		Yi et al. [86]
2015	Canada	Breast milk	55	GC-MS/MS	0.30 μg/L	5	<LOD-1.9 μg/L	<LOD		Arbuckle et al. [81]
2015	Canada	Infant formula	23	GC-MS	0.3 μg/L	30	<LOD-9.0 μg/L	<LOD (0.2)		Arbuckle et al. [81]

[a] 10th–95th

[b] The unit is μg/g creatinine

Cre. creatinine-adjusted concentration, *LOD* limit of detection, *Con* concentration, *GM* geometric mean, *SD* standard deviation

BPA exerts both estrogenic and antiandrogenic activity by interacting with ERα/ERβ and by antagonizing the AR, thereby eliciting effects on several target tissues through ER-dependent nuclear pathways [28, 29]. BPA can also act through various physiological receptors, such as the AR, aryl hydrocarbon receptor, peroxisome proliferator-activated receptor γ, and thyroid hormone receptor, leading to pleiotropic BPA behavior in the androgen-dependent systems, thyroid hormone function, the central nervous system, and the immune system [1, 2, 5, 92–98]. Based on a comprehensive review of in vivo and in vitro studies published from 2007 to 2013 regarding BPA toxicity and the adverse effects of BPA on fertility and reproductive function, BPA was identified as a reproductive toxicant, and its potential adverse effects are summarized as follows. BPA affected the onset of meiosis in fetal ovary and germ cell nest breakdown, altered steroidogenesis, disrupted ovary and uterine function, increased implantation failure, damaged testicular cells, reduced sperm production and quality, and perturbed hormone levels [3]. Ana Soto administered BPA perinatally and determined that BPA is a mammary carcinogen [99]. With the increased incidences of metabolic disorders, such as obesity and diabetes, BPA has also been suspected to be an obesogen [4, 35, 36].

Recently, epigenetic changes caused by BPA are drawing increasing attention. Epigenetics refers to heritable and stable changes in gene expression that do not involve alterations in DNA sequences [100]. BPA-related in vitro and in vivo epigenetic changes have been reported; they include DNA methylation, microRNA modulation, and differential histone modifications [101–105]. Considering that BPA may exhibit transgenerational effects through epigenetic mechanisms, early exposure to BPA may influence physiological expression and individual susceptibility across generations [106, 107]. Gestational exposure to BPA promotes epigenetic transgenerational inheritance, including alterations through DNA methylation in the germ line and epigenetic changes in the brain [106, 108]. Developmental gene expression alterations as a consequence of in utero BPA exposure have been demonstrated, suggesting that epigenetic regulation may be critical for fetal and postnatal health [109, 110].

Nonmonotonic dose–response effects and sexually dimorphic effects of BPA exposure with regard to receptor selectivity and competition have been reported; the responses are associated with specific dose ranges. Studies on its molecular mechanisms have revealed that BPA can bind to the ER, AR, or thyroid hormone receptor, resulting in nonmonotonic dose–response curves [111, 112]. For instance, an ex vivo study compared the response of 17-beta-estradiol (E2) and BPA in fetal mammary gland culture and noted that E2 inhibits ductal development and BPA exhibits a nonmonotonic dose–response effect: increased ductal development was observed in dams exposed to low BPA doses, whereas significantly decreased development was noted after exposure to high BPA doses [99]. Another study revealed an inverted U-shaped dose–response curve in body weight for exposure of female mice to low and high BPA doses. However, in male mice, this effect on body weight was observed only at high BPA doses [113]. Thus, the differences in exposure profiles and characteristics may account for the inconsistent results in epidemiological studies.

16.2.5 Effects on Human Health

A large number of studies links BPA to adverse health effects in animal studies and in vitro models; however, there is less research in human studies. Exposure to BPA is essentially ubiquitous in humans and BPA is detectable in most of the adults including pregnant women and children, which indicates that BPA is prevalent in utero [22, 79, 114]. According to the literature, approximately 70% of the human studies found significant adverse effects in non-occupational exposure which indicated that low-dose environmental exposure to BPA can cause adverse effects in general population [115]. Exposure to BPA during gestation could result in increased spontaneous abortion, abnormal gestation time, reduced birth weight, increased male genital abnormalities, and childhood obesity. In addition, early BPA exposure is suggested to be associated with disrupted neurodevelopment and altered behavior in children and also associated with increased risk of childhood asthma and wheeze. In this section, the link between prenatal BPA exposure in human and adverse health outcomes is reviewed.

Regarding birth weight, a fairly good number of literatures have been published; however, findings were inconclusive. Most of the studies used maternal urine samples for BPA exposure assessment and concluded that there was no significant association between prenatal exposure to BPA and birth weight [116–122]. Contrary, the studies using samples other than urine such as placenta and maternal blood seemed to conclude significant association between BPA levels and decreased birth weight or low birth weight [119–122]. These findings were based on different study design such as different exposure period, different specimens, varied sample size, and so on. The previous studies do not support clear-cut link between prenatal BPA exposure and altered birth weight.

Regarding neurodevelopment of children, higher levels of prenatal BPA exposure were reported to be associated with adverse outcomes of child neurobehavioral development. In previous studies, different test tools were used to assess child behavioral development including Bayley Scales of Infant Development 2nd Edition (BSID-II) [123], Behavior Assessment System for Children, Second Edition (BASC-2) [114, 124, 125], Child Behavior Checklist (CBCL) [123, 126–128], Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P) [114], Social Responsiveness Scale (SRS) [129, 130], The Conners' ADHD/DSM-IV Scales (CADS) [125], Strengths and Difficulties Questionnaire (SDQ) [131], and The NICU Network Neurobehavioral Scale (NNNS) [132]. Though some of these test tools may be correlated and behavioral outcomes are comparable, however, they do not necessarily measure the same neurobehavioral outcomes. Up to now, there has been no clear evidence to show which test tool provides more accurate measurements of child neurobehavior. The recent review concluded that early BPA exposure was associated with hyperactivity in boys and girls in human study [133]. In addition, time of exposure assessment varied among previous studies. Some of the studies found association between prenatal expo-

sure to BPA and adverse behavioral outcomes such as aggression, hyperactivity, anxiety, internalizing and externalizing problems, and depression [114, 123–128, 134–136], contrary, early childhood exposure to BPA was reported to be associated with problems such as inattention, anxiety, depression, and hyperactivity [125, 128, 134, 135]. Other studies, however, found no significant association between pre-/postnatal exposure to BPA and adverse behavioral outcomes [129–132, 137]. In previous studies, sex-specific effects of BPA exposure on behavioral outcomes have been reported; however, findings have been inconsistent. Some found adverse effects in girls [114, 124]; on the other hand, others found in boys [125–128, 136]. As BPA is an endocrine disruptor, it is possible that it has an impact on the development of sexually dimorphic brain structures prenatally. Alternation in brain structure could possibly be associated with the different behavioral outcomes that have been reported for boys and girls. Further research is warranted to clarify the influence of pre-/postnatal exposure to BPA on child long-term neurobehavioral outcomes.

Another developmental endpoint that has been studied in association with prenatal BPA exposure in epidemiological studies is childhood wheeze and asthma. Relationship between prenatal exposure to BPA and childhood wheeze, asthma, and allergic diseases have been reported from several cohort studies [138–142]. Most of the previous studies showed an increased risk of childhood wheeze in association with prenatal BPA exposure [140–142] and one study reported an increase in allergic disease in association with prenatal exposure to BPA only in female infants [138]. Contrary, one study reported an inverse association between prenatal BPA exposure and wheeze prevalence at 5 years of age [139]. The previous studies investigated prenatal exposure to BPA and childhood asthma did not find any significant association [139, 142]. Four of the studies examined early postnatal BPA exposure on prevalence of wheeze and asthma [139, 141, 143, 144]. Most of the studies suggested the risk of childhood wheeze and asthma increased with early childhood BPA exposure [139, 143, 144]. One study reported no association between child BPA exposure and concurrent and future wheeze [141]. There are important windows of immune development in which environmental exposures can either increase or decrease the risk of allergic disease [145].

When discussing health outcomes in association with BPA exposure, the number of BPA exposure measurements should be considered because BPA has very short half-life and rapidly excreted from the body. Ideally, BPA concentration should be measured more than once [146]. Other issue should be considered is that timing of exposure was usually different among studies and this may provide conflicting findings. In addition, exposure profile such as exposure levels should be considered as some of the animal studies indicated U-shaped association with health outcomes. Finally, BPA exposure levels were reported to be associated with certain characteristics such as socio-economic status and lifestyles [71, 147, 148]; thus, covariates used for statistical analyses should be considered in epidemiological studies.

16.3 Alkylphenols

16.3.1 Introduction

Alkylphenols (APs) are a group of degradation products derived primarily from the hydrolytic breakdown of their parent compounds (Fig. 16.3), AP ethoxylates (APEOs), which are the most widely used nonionic surfactants [149]. Structurally, an APEO is a phenol ring with alkyl and ethoxylate (EO) chains. Nonylphenol (NP) EOs (NPEOs) and octylphenol (OP) EOs (OPEOs) account for approximately 80% and 20% of APEOs in the world market, respectively [150, 151]. APEOs, which exhibit both hydrophilic and hydrophobic properties, are used as detergents, emulsifiers, and dispersive agents. NP can be used to produce tris(4-nonyl-phenyl) phosphite, which acts as an antioxidant and stabilizer in certain plastics, such as polyethylene (PE), polyethylene terephthalate (PET), and polyvinyl chloride (PVC). These plastics are used in food containers, milk and other beverage jugs, wrapping films, medical products, and children's toys [152–156]. Phenol reacts with nonene isomers, and from this reaction, various branched NP isomers have been produced. The most common among these isomers is para-NP. Technical NP contains several Para substituted isomers [157, 158]. According to the estimate in the NP and NPEOs Action Plan ([RIN 2070-ZA09], /18/2010) issued by the United States Environmental

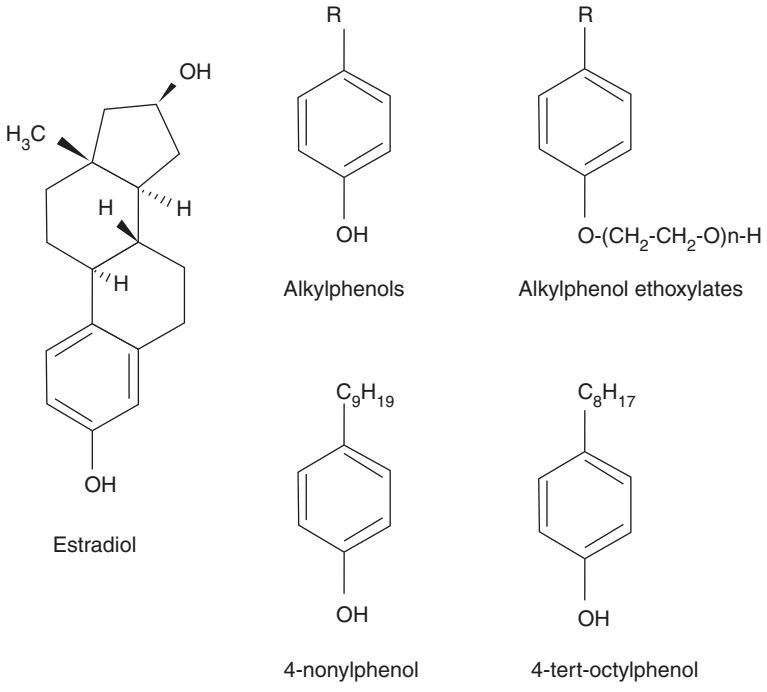


Fig. 16.3 Structure of APs

Protection Agency, demand for NP was 380 million pounds in 2010. The consumption of NPEOs surfactants in the USA and Canada was 300–400 million pounds/year [159]. NP is also the most commercially critical AP in Europe, where approximately 75,000 tons of NP is produced annually [160]. The intensive use of APEOs results in their entry into environmental media, where they biodegrade to derivatives with shorter chains, such as AP monoethoxylates (AP1EO), AP diethoxylates (AP2EO), alkylphenoxyacetic acids (AP1EC), alkyphenol monoethoxy acetic acid (AP2EC), and alkylphenoxyethoxyacetic acid (APEC2), and then finally back into APs [161, 162]. APEOs with fewer EO groups are considered to be relatively lipophilic and hydrophobic, and these properties are conducive to biomagnification and bioaccumulation, whereby the APEOs spread through the ecological food chain [163–165]. Commonly consumed food products may be contaminated with APs because of bioaccumulation and transfer through food chains, contamination in food processing, and migration from packaging materials [149, 153, 166–171]. According to a recent review, NP concentration in various foods was 0.1–100 μg/kg fresh weight (f.w.) [172]. Human exposure to APs is widespread, mainly resulting from ingestion of AP-contaminated water and food, particularly seafood, which has high NP concentrations [149, 168, 171]. Increasing concerns regarding the potential estrogenic activity of APs were validated in 1991 when Sato et al. observed that NP caused proliferation of human MCF-7 breast tumor cells and triggered mitotic activity in rat endometrium [156]. The estrogenic properties of APs may cause related adverse effects in ecosystems in vivo and ex vivo, including lesions in gonadal development, low mass of testes and epididymis, inhibition of ovarian development, and low viability of offspring [173–181]. In 2003, the European Union proposed to restrict the marketing and major use of NP and NPEOs to control potential related negative effects on human health. The Danish Institute of Safety and Toxicology proposed a TDI of 5 μg/kg BW/day for NP.

16.3.2 Metabolism of APs

The mechanism underlying AP metabolism has been identified in many animal studies (Fig. 16.4). One study indicated that ingested APs were rapidly absorbed in the gastrointestinal tract and metabolized by glucuronation and sulfation in the liver [182]. The major glucuronide conjugation of APs occurred both in the aromatic hydroxyl group and hydroxylated alkyl chain, and glucuronide conjugate of APs, glucuronide conjugates of ring, or side-chain hydroxylated APs were formed [183, 184]. In the mammalian liver, the aromatic ring of APs was hydroxylated to catechol structures and formed glucuronide conjugates of the catechol metabolites [185]. Another metabolic pathway, catalyzed by sulfotransferase, involved sulfated conjugates of hydroxylated phenols [46]. In the liver tissue, the alkyl chain of conjugated APs was shortened and transported by multidrug resistance-associated protein 2 (MRP-2), and then excreted through bile and venous blood [186]. In vivo, the main metabolic route of APs resulted in AP elimination through feces [182, 187].

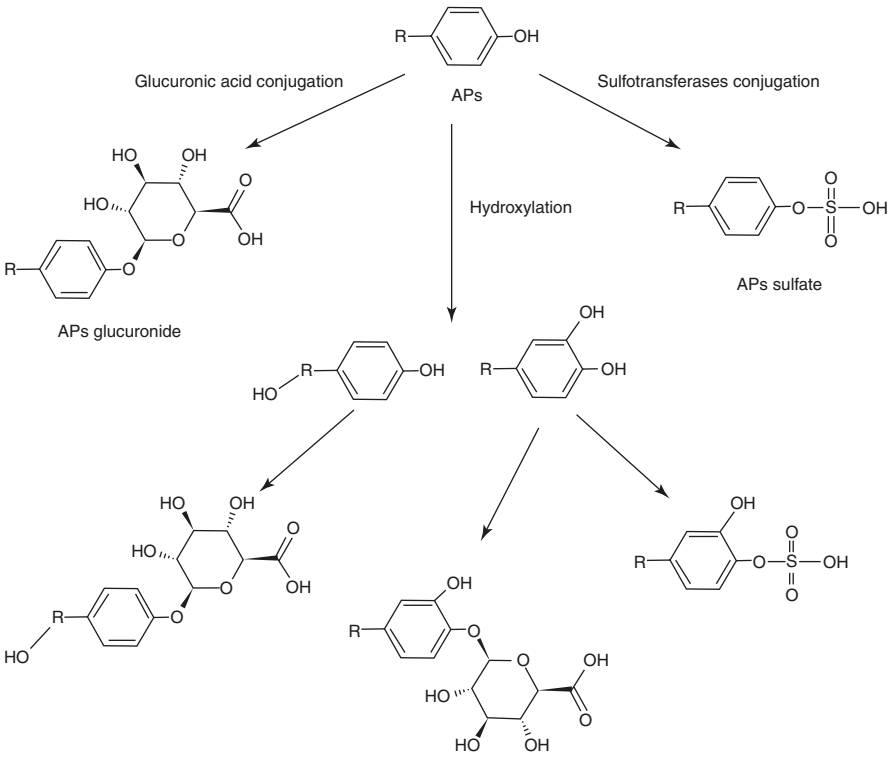


Fig. 16.4 Proposed biotransformation pathways of APs

In rats, placental transfer of NP in offspring was observed; in this phenomenon, NP was present in the forms of active aglycone and conjugated NP [188]. In humans, APs were mainly eliminated in urine. Pharmacokinetic behavior of NP in human volunteers was noted, and the highest blood NP level was reached within 1 h of oral or intravenous administration. Bioavailability after oral application was approximately 20%, and parent and conjugated NP was eliminated within 8 h. NP levels in urine and feces only accounted for approximately 10% of the administered dose, indicating that additional metabolites were present or that the NPs may have accumulated in the human body [189].

16.3.3 Exposure Assessment

APs, mainly NP, are ubiquitous in environmental matrices and distributed extensively in wastewater effluent, river water, and sediment [154, 164, 190, 191]. A national survey of APEOs in the US sewage sludge revealed that NP was the most abundant AP, and the mean annual load of NP compounds was 2408–7149 metric

tons [190]. In France, NP, NP1EC, and NP2EO were prevalent in raw water, and NP was the most concentrated AP in treated water [192]. Similarly, NP was the most prevalent AP in the East China Sea [193]. The tendency of NP to adsorb on to sediments and its low levels of degradation result in accumulation of the compound in marine organisms and wildlife followed by its spread throughout the ecological food chain [163–165, 194]. Lee et al. (2015) estimated that the bioconcentration factors and biota-sediment accumulation factors of NP in wild freshwater fish were (74.0–2.60) × 10^4 L/kg and 0.003–18.3, respectively, and indicated the adverse effects of NP on aquatic organisms [195].

The main sources of human exposure to APs are contaminated food and drinking water [149, 153, 168, 171, 192, 196]. In Germany, Guenther et al. identified NP in 39 commercially available foodstuffs and reported that NP was ubiquitous in each food. In terms of the fresh weight of the food, NP concentrations varied between 0.1 and 19.4 μg/kg, regardless of the food's fat content [149]. In Taiwan, 25 commonly available foodstuffs were analyzed based on 318 samples. NP was identified in concentrations ranging from 11.7 to 399.55 ng/g wet, and OP was identified in concentrations ranging from undetectable to 152.15 ng/g wet. The most commonly consumed sources of NP in order according to prevalence of consumption were rice (21%), aquatic products (18%), and livestock (17%). The highest NP concentration was detected in oysters [168]. In China, NP was detected in 99% of foodstuff samples at concentrations of 30 ng/kg to 1268 μg/kg [197]. Other studies have reported NP concentrations of 5–50 μg/kg f.w. for vegetables and fruits [198–200]. Adult intake of NP has been estimated to be 0.067–0.52 μg/kg BW/day [149, 168, 197–200]. Moreover, APs were detected in breast milk at concentrations of 0.07–57.3 ng/mL [149, 169, 201–203]. The maximum concentrations of NP and OP that have been identified in baby formula are 17.1 μg/kg and 616 ng/kg, respectively. The estimated median daily intake of NP and OP is respectively 0.01–0.08 μg/kg BW and 0.6–2.7 ng/kg BW among babies and 0.04–0.05 μg/kg BW and 2.3–3.9 ng/kg BW among toddlers [169]. The migration of APs from materials used for food packaging or processing into foods is a major cause of AP ingestion [153, 195, 204, 205]. In 60 rubber products tested in Japan, OP and NP concentrations were 2.2–37 and 2.6–513 μg/g, respectively [204]. In bottled water or milk surrogates, the highest levels of NP were identified in HDPE (180 ng/L) and PVC (300 ng/L) jugs [205]. In a simulation of food refrigeration at 5 °C for 24 h, 2.9–6.4% migration of NP from PVC stretch films into vegetable and fruit was recorded [206]. Fernades et al. analyzed 25 food-contact materials and indicated that the NP concentrations were 64–287 μg/g in polystyrene (PS) and PVC samples [196].

APs have been detected in various biological matrices, such as urine, blood, amniotic fluid, breast milk, and placenta (Table 16.2). Biomonitoring of textile and housekeeping workers for occupational AP exposure revealed that the average plasma NP and OP concentrations in the housekeeping workers were 53.21 ± 49.74 and 16.02 ± 2.81 ng/g, respectively [213]. In the general population, the mean urinary NP and OP concentrations were 0.1–3.7 μg/L and 0.6–3.2 ng/mL, respectively [22, 79, 207, 209, 214, 215]. The ability of NP to cross the human placenta was confirmed in a dual ex vivo recirculating model of placental perfusion, and NP has

Table 16.2 APs levels in various biospecimens

APs	Year	Country	Specimen	*N*	Detection method	LOD	Detection rate (%)	Con. range	GM (median)	Mean ± SD	Reference
NP	2005	USA	Adult urine	394	GC-MS	0.1 μg/L	51	95th: 1.4 μg/g cre.	(<0.1)		Calafat et al. [114]
	2009	Taiwan	Children urine	786	HPLC-FLD	1.6 μg/L	30	<LOD-178.3 μg/g cre.	1.27[a]		Chen et al. [207]
	2014	Taiwan	Maternal urine	162	HPLC-FLD	0.20 ng/mL	97	0.04–48.45 μg/g cre.	4.10 (4.50)[a]		Chang et al. [208]
	2015	China	Maternal urine	137	GC-MS	0.12 ng/mL	87	<LOD-160.2 μg/g cre.	3.4 (3.9)[a]		Liu et al. [70]
	2017	Korea	Adult urine	1865	GC-MSD	0.05 ng/mL	83	<LOD-4477.0 ng/mL	3.70 (8.10)		Park et al. [209]
	2013	China	Maternal serum	201	HPLC-ESI-MS/MS	0.12 mg/L	66	<LOD-5.58 mg/L	(0.38)		Li et al. [210]
	2017	India	Maternal plasma	40	GC-MS	0.54 ng/mL	90		7.61 (9.38)		Sudhanshu et al. [83]
	2017	India	Amniotic fluid	40	GC-MS	0.54 ng/mL	88		7.79 (8.44)		Sudhanshu et al. [83]
	2003	Malaysia	Fetal cord plasma	180	GC-MS	0.05 ng/mL	86	0.05–15.17 ng/mL			Tan et al. [67]
	2013	China	Fetal cord serum	201	HPLC-ESI-MS/MS	0.04 mg/L	76	<LOD-1.28 mg/L	(0.28)		Li et al. [210]
	2014	Taiwan	Fetal cord plasma	29	HPLC-FLD	1.87 ng/mL	100	4.4–57.6 ng/mL	18.8	22.4 ± 13.8	Huang et al. [202]
	2014	Taiwan	Placenta	30	HPLC-FLD	1.87 ng/g	100	5.4–54.4 ng/g	19.8	23.6 ± 13.2	Huang et al. [202]
	2003	Japan	Breast milk	3	GC-MS	0.50 ng/g	100	0.65–1.40 ng/g		1.05	Otaka et al. [211]
	2008	Italy	Breast milk	10	GC-MS	9.8 ng/mL	100	13.4–56.3 ng/mL		32.00 ± 16.2	Ademollo et al. [201]
	2010	Taiwan	Breast milk	59	GC-MS	0.3 ng/g	86		2.26	4.47 ± 4.31	Chen at al [212]
	2013	Korea	Colostrum	326	LC-MS/MS		16	<LOD-23.4 ng/mL			Yi et al. [86]
	2017	Turkey	Breast milk	100	HPLC-FLD	0.26 ng/mL		0.26–47.5 ng/mL	5.01 (8.46)	10.1 ± 0.98	Sise et al. [203]

OP	2008	USA	Urine	2517	HPLC-APCI-MS/MS	0.2 μg/L	57	0.2–20.6 μg/L	(0.3)		Calafat et al. [22]
	2013	China	Maternal urine	567	UPLC-MS/MS	0.02 ng/mL	15	<LOD-2.53 ng/mL			Tang et al. [117]
	2015	China	Maternal urine	137	GC-MS	0.24 ng/mL	70	<LOD-33.7 μg/g cre.	1.1 (1.1)[a]		Liu et al. [70]
	2017	Korea	Adult urine	1865	GC-MSD	0.05 ng/mL	92	<LOD-988.7 ng/mL	0.60 (0.77)		Park et al. [209]
	2013	China	Maternal serum	201	HPLC-ESI-MS/MS	0.20 mg/L	64	<LOD-2.97 mg/L	(0.47)		Li et al. [210]
	2017	India	Maternal plasma	40	GC-MS	0.63 ng/mL	63		3.69 (5.46)		Sudhanshu et al. [83]
	2017	India	Amniotic fluid	40	GC-MS	0.63 ng/mL	50		3.10 (5.72)		Sudhanshu et al. [83]
	2003	Malaysia	Fetal cord plasma	180	GC-MS	0.05 ng/mL	53	0.05–4.17 ng/mL			Tan et al. [67]
	2013	China	Fetal cord serum	201	HPLC-ESI-MS/MS	0.08 mg/L	74	<LOD-1.25 mg/L	(0.40)		Li et al. [210]
	2006	USA	Breast milk	20	LC-MS/MS	2.55 ng/mL	25	<LOD-7.6 ng/mL		2.7	Ye et al. [85]
	2008	Italy	Breast milk	10	GC-MS	0.019 ng/mL	70	<LOD-0.21 ng/mL		0.12 ± 0.07	Ademollo et al. [201]
	2010	Taiwan	Breast milk	59	GC-MS	0.01 ng/g	31		0.02	1.29 ± 1.68	Chen at al [212]
	2013	Korea	Colostrum	326	LC-MS/MS		28	<LOD-30.9 ng/mL			Yi et al. [86]

[a]The unit is μg/g creatinine; *Cre.* creatinine-adjusted concentration, *LOD* limit of detection, *Con* concentration, *GM* geometric mean, *SD* standard deviation

been detected in amniotic fluid, umbilical cord blood, and placenta [83, 202, 216, 217]. In India, the concentrations of NP and OP were respectively 9.38 and 5.46 ng/mL in the maternal blood and 8.44 and 5.72 ng/mL in the amniotic fluid [83]. The level of NP in placenta was 5.4–54.4 ng/g [202]. Among the umbilical cord blood samples, higher NP concentration was observed in umbilical venous plasma than in umbilical arterial plasma, indicating that fetuses could be exposed to high levels of NP from transplacental absorption and partitioning between the maternal and fetal compartments, as a consequence of the developing fetus's poor detoxification mechanisms [217].

16.3.4 Effects of APs In Vitro and In Vivo

The potential of APs for estrogenic activity has been discovered and confirmed in vitro and in vivo [156, 218–223]. APs can affect the function of the endocrine system by interacting with ER and disrupting the normal signal pathways. Soto et al. discovered that NP induced cell proliferation and bonded to ER in human estrogen-sensitive MCF7 breast tumor cells [156]. NP exhibited the ability to compete with estradiol and promegestone in binding to estrogen and progesterone receptors [218]. Many in vivo studies have demonstrated that APs can induce male vitellogenin, which is normally secreted by the liver of female fish in response to endogenous estrogens [220–222]. Gray and Metcalfe (1997) discovered that Japanese male *medaka* developed testis–ova after exposure to NP; this intersex condition is characterized by the presence of both testicular and ovarian tissue in the gonads [223]. The toxicity and estrogenic potency of APs and APEOs depend on the length and structure of the alkyl chain and the number of EO groups. According to a yeast screen assay, the most estrogenically potent APs have 6–8-carbon-long alkyl chains with tertiary branching, and these chains are attached to the phenolic ring in the para position [219].

As an estrogen-mimicking compound, NP can affect hormonal homeostasis; potentially affected process includes sex hormone synthesis and thyroid hormone regulation [224–229]. NP-treated *Clarias gariepinus* exhibited decreased thyroid-stimulating hormone (TSH), triiodothyronine (T3), total thyroxine (T4), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) as well as increased testosterone concentrations and E2 levels [224]. NP also exhibited a nonmonotonic dose–response and a sexually dimorphic effect. Furthermore, NP inhibited the production of progesterone and androstenedione, but stimulated progesterone release at low doses [228, 229]. Findings regarding the compound's effects on testosterone synthesis have varied: NP stimulated testosterone release through increase in both protein levels and activities of steroidogenic acute regulatory and cytochrome P450 side-chain cleavage proteins; however, it also inhibited human chorionic gonadotropin-induced testosterone release in rat Leydig cells [226]. In immature male yellowfin seabream, E2 levels increased with low-dose NP injections but decreased with high doses [225].

In vivo studies have revealed that AP administration to pregnant or neonatal animals influences regulation of inflammation and redox homeostasis, leading to reproductive toxicity, neurotoxicity, and genotoxicity. Maternal NP exposure may stimulate Akt/mitogen-activated protein kinase/activator protein 1 signaling in microglia, leading to increased and production of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α in offspring hippocampus [230, 231]. Elevated levels of these inflammatory cytokines may be associated with the risks of abortion, dystocia, and adverse delivery outcomes [232]. AP-induced oxidative stress can cause DNA damage and lipid peroxidation [233, 234]. In vitro, AP exposure increased the production of reactive oxygen species (ROS), nitric oxide (NO), and peroxynitrated protein derivatives. The oxidative stress and lipid peroxidation induced by APs may be related to the inhibition of antioxidant enzymes or mitochondrial electron transport chain complexes [233–236]. Intracellular accumulation of ROS in cells may influence hormonal profile and cell viability and induce apoptosis and DNA damage [237–240]. Moreover, excessive ROS in spermatozoa may impair spermatogenesis and sperm function [233, 241]. Neurological damage and impaired brain function associated with NP have also been examined. NP may alter the activity of acetylcholine esterase and choline acetyltransferase in the hippocampus, resulting in memory impairment, anxiety, and learning ability decline [242–245]. Finally, NP may also affect adipogenesis and fatty acid synthesis, leading to glucose metabolism disorder [246–249].

16.3.5 Effects of APs on Human Health

Prenatal exposure to APs can possibly be associated with adverse health outcomes; however, not as many epidemiological studies have been reported as prenatal exposure to BPA and its adverse health outcome in human. Among various APs, most of the previous epidemiological studies focused on triclosan (TCS), 3-benzophenone (BP-3), 2,4-dichlorophenol (2,4-DCP), 2,5-dichlorophenol (2,5-DCP). In this section, these APs were reviewed in association with adverse health outcomes.

Regarding birth outcomes including birth size, gestational age, and anogenital distance (AGD), most of the previous literatures reported prenatal exposure to APs and birth weight. For BP-3, prenatal exposure was reported to be associated with increased birth weight particularly among boys [116, 118, 250], but not among girls [118]. Contrary for TCS, decreased birth weight has been reported from the prospective studies [250–252], except one study which reported increased birth weight among girls [253]. Similarly, for 2,4-DCP and 2,5-DCP, decreased birth weight among boys has been reported [116, 251, 252]. One study from agricultural region in China reported that maternal urine levels of 2,4,6-trichlorophenol (2,4,6-TCP) and pentachlorophenol (PCP) were associated with lower birth weight among boys while head circumference was associated with 2,4-DCP and 2,5-DCP only among females [254]. Both positive and inverse associations have been reported between prenatal BP-3 and TCS exposures and gestational age [219, 255]. Meanwhile,

studies have reported no significant association of birth outcomes with prenatal exposure to TCS [71, 256–258]. Overall, associations between prenatal exposure to APs and birth outcomes were not clearly understood and there is a need for further studies.

Other health outcomes including hormone levels in cord blood or newborns, childhood adiposity and growth, pubertal timing, and neurobehavioral development have been reported from prospective studies in association with prenatal exposure to APs. One study in China reported that prenatal exposure to TCS was associated with increased testosterone and decreased estradiol in cord blood [259]. A study from the US agricultural region reported no association between maternal urinary TCS, BP-3, 2,4-DCP, and 2,5-DCP and newborn's thyroid hormone levels [260]. Overall, there seems to be not enough evidence on disruption of hormone levels of newborn in association with prenatal exposure to APs and epidemiological studies, especially investigation in prospective birth cohort studies are warranted. One recent article introduced later pubic hair development in association with prenatal exposure to 2,5-DCP and earlier menarche with prenatal TCS and 2,4-DCP exposure among girls [261]. In this study, exposure levels of APs were higher compared to the other prospective studies. Three studies in the USA have reported childhood adiposity in association with prenatal exposures to APs. One study found that maternal third trimester urinary BP-3 levels were inversely associated with percent fat mass in girls at school ages; however, TCS and 2,5-DCP levels were not associated [262]. Another study found weak positive associations between second trimester urinary TCS concentrations and weight at ages 1, 2, and 3 years only among boys [116]. The other study found no association between maternal urinary concentrations of TCS during pregnancy and childhood adiposity at age 8 years [263]. Findings from these studies were inconsistent and this could be due to different exposure assessment timing, different outcome measurement timing. To elucidate the association between prenatal exposure to APs and childhood growth including pubertal development and adiposity, further studies are necessary.

In the past few years, studies that investigated association between prenatal exposure to TCS, BP-3, 2,4-DCP, and 2,5-DCP and child neurobehavioral development have been published; however, findings were controversial. A study in France investigated associations between maternal urinary concentrations of TCS, BP-3, 2,4-DCP, and 2,5-DCP and child neurobehavioral development such as intelligence and behavioral problems only among boys [264, 265]. They reported that TCS tended to be positively associated with emotional symptoms at ages 3 and 5 years [264]; however, none of the APs was associated with verbal and performance IQ among boys at age 5 years [265]. The other study in the USA found no association between maternal urinary TCS levels and visual-spatial abilities at 8 years of age [266]. Similarly, a study in Canada reported that urinary TCS concentrations in early pregnancy were not adversely associated with child neurobehavioral development at age 3 years [267]. Based on these previous studies, exposure to TCS during pregnancy seemed to be not significantly associated with child neurobehavioral development; however, these studies were conducted in

Europe and North American countries, thus more evidence from other regions such as Asia, South America, and African countries should be accumulated and should further be investigated.

Regarding childhood asthma, wheeze, and allergic disease in association with prenatal exposure to APs, only a few studies have been published. A study in France found that increased levels of 2,5-DCP tended to be associated with altered respiratory health. In addition, they found that reduced rates of bronchiolitis/bronchitis and wheezing with increased exposure to BP-3 tended to reduce rates of bronchiolitis/bronchitis at 5 years of age [268]. A study in the USA found associations of higher 2,5-DCP urinary concentrations with parent-reported respiratory and allergic outcomes among boys but not among girls and associations of BP-3 concentrations with reduced odds of wheeze symptoms, particularly among girls at ages 6–7 years [269]. To date, there were only two studies that examined associations between maternal urinary concentrations of APs during pregnancy and childhood asthma, wheeze, and allergic diseases in prospective cohort studies. The other study in the USA assessed childhood asthma, recurrent wheeze, and food or environmental sensitization at age 3 years in association with TCS concentrations in maternal plasma and found no statistically significant associations [270]. One other prospective study in Canada assessed association between prenatal exposure to TCS and immunoglobulin E (IgE), thymic stromal lymphopoietin (TSLP), and interleukin-33 (IL-33) and observed no statistically significant associations between prenatal TCS concentrations and elevated concentrations of any immune system biomarker [271]. With limited evidences from these studies, again TCS exposure during pregnancy may not seem to adversely influence on childhood asthma, wheeze, and allergic diseases; however, findings on BP-3 and 2,5-DCP exposure during pregnancy have been inconsistent. Besides, sex-specific influence has also been reported. Needless to say, further studies are warranted.

16.4 Conclusions

This chapter summarizes the exposure assessment and related potential health risks of bisphenols and alkylphenols. BPA, NP, OP, TCS, BP-3, 2,4-DCP, and 2,5-DCP have been proven as endocrine-disrupting chemicals and exhibited potential health effects on susceptible pregnant women, fetus, and children. Food intake contaminated by these substances is the main human exposure source. Despite the fact that laws and regulations were established to restrict the production and marketing of certain bisphenols and alkylphenols, the increasing concerns regarding repeated and consistent low-dose exposure are addressed. Besides, the coexposure, individual susceptibility, and non-linear dose response effect could be the challenge in epidemiological studies with inconsistent findings. Future perspective of research is recommended toward “exposome” and utilizes advanced statistical analysis to comprehensively evaluate the multitude of environmental influences and human exposures.

References

1. MacLusky NJ, Hajszan T, Leranth C. The environmental estrogen bisphenol A inhibits estradiol-induced hippocampal synaptogenesis. Environ Health Perspect. 2005;113(6):675–9.
2. Miyatake M, et al. Dynamic changes in dopaminergic neurotransmission induced by a low concentration of bisphenol-A in neurones and astrocytes. J Neuroendocrinol. 2006;18(6):434–44.
3. Peretz J, et al. Bisphenol A and reproductive health: update of experimental and human evidence, 2007-2013. Environ Health Perspect. 2014;122(8):775–86.
4. Mirmira P, Evans-Molina C. Bisphenol A, obesity, and type 2 diabetes mellitus: genuine concern or unnecessary preoccupation? Transl Res. 2014;164(1):13–21.
5. Miller MD, et al. Thyroid-disrupting chemicals: interpreting upstream biomarkers of adverse outcomes. Environ Health Perspect. 2009;117(7):1033–41.
6. Brede C, et al. Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing. Food Addit Contam. 2003;20(7):684–9.
7. Vandenberg LN, et al. Human exposure to bisphenol A (BPA). Reprod Toxicol. 2007;24(2):139–77.
8. Jiao FR, Sun XJ, Pang ZT. Production and market analysis of bisphenol A. Chem Ind. 2008;26(9):21.
9. Cao XL, Corriveau J, Popovic S. Levels of bisphenol A in canned soft drink products in Canadian markets. J Agric Food Chem. 2009;57(4):1307–11.
10. Cao XL, Corriveau J, Popovic S. Bisphenol A in canned food products from Canadian markets. J Food Prot. 2010;73(6):1085–9.
11. Bjornsdotter MK, de Boer J, Ballesteros-Gomez A. Bisphenol A and replacements in thermal paper: a review. Chemosphere. 2017;182:691–706.
12. Matsumoto H, Adachi S, Suzuki Y. Bisphenol A in ambient air particulates responsible for the proliferation of MCF-7 human breast cancer cells and its concentration changes over 6 months. Arch Environ Contam Toxicol. 2005;48(4):459–66.
13. Suzuki T, et al. Environmental fate of bisphenol A and its biological metabolites in river water and their xeno-estrogenic activity. Environ Sci Technol. 2004;38(8):2389–96.
14. Hashimoto S, et al. Horizontal and vertical distribution of estrogenic activities in sediments and waters from Tokyo Bay, Japan. Arch Environ Contam Toxicol. 2005;48(2):209–16.
15. Kawahata H, et al. Endocrine disrupter nonylphenol and bisphenol A contamination in Okinawa and Ishigaki Islands, Japan—within coral reefs and adjacent river mouths. Chemosphere. 2004;55(11):1519–27.
16. Kuch HM, Ballschmiter K. Determination of endocrine-disrupting phenolic compounds and estrogens in surface and drinking water by HRGC-(NCI)-MS in the picogram per liter range. Environ Sci Technol. 2001;35(15):3201–6.
17. Corrales J, et al. Global assessment of bisphenol A in the environment: review and analysis of its occurrence and bioaccumulation. Dose Response. 2015;13(3):1559325815598308.
18. Geens T, et al. A review of dietary and non-dietary exposure to bisphenol-A. Food Chem Toxicol. 2012;50(10):3725–40.
19. Chen WY, Shen YP, Chen SC. Assessing bisphenol A (BPA) exposure risk from long-term dietary intakes in Taiwan. Sci Total Environ. 2016;543(Pt A):140–6.
20. Volkel W, et al. Metabolism and kinetics of bisphenol A in humans at low doses following oral administration. Chem Res Toxicol. 2002;15(10):1281–7.
21. Stahlhut RW, Welshons WV, Swan SH. Bisphenol A data in NHANES suggest longer than expected half-life, substantial nonfood exposure, or both. Environ Health Perspect. 2009;117(5):784–9.
22. Calafat AM, et al. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. Environ Health Perspect. 2008;116(1):39–44.
23. Maffini MV, et al. Endocrine disruptors and reproductive health: the case of bisphenol-A. Mol Cell Endocrinol. 2006;254-255:179–86.

24. Koda T, Morita M, Imai H. Retinoic acid inhibits uterotrophic activity of bisphenol A in adult ovariectomized rats. J Nutr Sci Vitaminol (Tokyo). 2007;53(5):432–6.
25. Weinhouse C, et al. Dose-dependent incidence of hepatic tumors in adult mice following perinatal exposure to bisphenol A. Environ Health Perspect. 2014;122(5):485–91.
26. Macczak A, et al. The in vitro comparative study of the effect of BPA, BPS, BPF and BPAF on human erythrocyte membrane; perturbations in membrane fluidity, alterations in conformational state and damage to proteins, changes in ATP level and Na+/K+ ATPase and AChE activities. Food Chem Toxicol. 2017;110:351–9.
27. Huc L, et al. Low concentrations of bisphenol A induce lipid accumulation mediated by the production of reactive oxygen species in the mitochondria of HepG2 cells. Toxicol In Vitro. 2012;26(5):709–17.
28. Gould JC, et al. Bisphenol A interacts with the estrogen receptor alpha in a distinct manner from estradiol. Mol Cell Endocrinol. 1998;142(1–2):203–14.
29. Andersen HR, et al. Comparison of short-term estrogenicity tests for identification of hormone-disrupting chemicals. Environ Health Perspect. 1999;107(Suppl 1):89–108.
30. Ashby J, Tinwell H. Uterotrophic activity of bisphenol A in the immature rat. Environ Health Perspect. 1998;106(11):719–20.
31. Tyl RW, et al. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol Sci. 2002;68(1):121–46.
32. Rubin BS, et al. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect. 2001;109(7):675–80.
33. Savabieasfahani M, et al. Developmental programming: differential effects of prenatal exposure to bisphenol-A or methoxychlor on reproductive function. Endocrinology. 2006;147(12):5956–66.
34. Kim JC, et al. Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy. Life Sci. 2001;69(22):2611–25.
35. Boucher JG, et al. In vitro effects of bisphenol A beta-D-glucuronide (BPA-G) on adipogenesis in human and murine preadipocytes. Environ Health Perspect. 2015;123(12):1287–93.
36. Legeay S, Faure S. Is bisphenol A an environmental obesogen? Fundam Clin Pharmacol. 2017;31(6):594–609.
37. Wu LH, et al. Occurrence of bisphenol S in the environment and implications for human exposure: a short review. Sci Total Environ. 2018;615:87–98.
38. Rochester JR, Bolden AL. Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol A substitutes. Environ Health Perspect. 2015;123(7):643–50.
39. Liao C, et al. Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure. Environ Sci Technol. 2012;46(16):9138–45.
40. Song S, et al. Occurrence and profiles of bisphenol analogues in municipal sewage sludge in China. Environ Pollut. 2014;186:14–9.
41. Yang Y, et al. Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr A. 2014;1328:26–34.
42. Moreman J, et al. Acute toxicity, teratogenic, and estrogenic effects of bisphenol A and its alternative replacements bisphenol S, bisphenol F, and bisphenol AF in zebrafish embryo-larvae. Environ Sci Technol. 2017;51(21):12796–805.
43. Gramec Skledar D, Peterlin Masic L. Bisphenol A and its analogs: do their metabolites have endocrine activity? Environ Toxicol Pharmacol. 2016;47:182–99.
44. Siracusa JS, et al. Effects of bisphenol A and its analogs on reproductive health: a mini review. Reprod Toxicol. 2018;79:96–123.
45. Hanioka N, Naito T, Narimatsu S. Human UDP-glucuronosyltransferase isoforms involved in bisphenol A glucuronidation. Chemosphere. 2008;74(1):33–6.
46. Suiko M, Sakakibara Y, Liu MC. Sulfation of environmental estrogen-like chemicals by human cytosolic sulfotransferases. Biochem Biophys Res Commun. 2000;267(1):80–4.

47. Ye X, et al. Quantification of urinary conjugates of bisphenol A, 2,5-dichlorophenol, and 2-hydroxy-4-methoxybenzophenone in humans by online solid phase extraction-high performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2005;383(4):638–44.
48. Provencher G, et al. Determination of bisphenol A, triclosan and their metabolites in human urine using isotope-dilution liquid chromatography-tandem mass spectrometry. J Chromatogr A. 2014;1348:97–104.
49. Pritchett JJ, Kuester RK, Sipes IG. Metabolism of bisphenol A in primary cultured hepatocytes from mice, rats, and humans. Drug Metab Dispos. 2002;30(11):1180–5.
50. Zalko D, et al. Biotransformations of bisphenol A in a mammalian model: answers and new questions raised by low-dose metabolic fate studies in pregnant CD1 mice. Environ Health Perspect. 2003;111(3):309–19.
51. Ye X, et al. In-vitro oxidation of bisphenol A: is bisphenol A catechol a suitable biomarker for human exposure to bisphenol A? Anal Bioanal Chem. 2011;399(3):1071–9.
52. Atkinson A, Roy D. In vivo DNA adduct formation by bisphenol A. Environ Mol Mutagen. 1995;26(1):60–6.
53. Balakrishnan B, et al. Transfer of bisphenol A across the human placenta. Am J Obstet Gynecol. 2010;202(4):393-e1–7.
54. Nishikawa M, et al. Placental transfer of conjugated bisphenol A and subsequent reactivation in the rat fetus. Environ Health Perspect. 2010;118(9):1196–203.
55. Cappiello M, et al. Uridine 5′-diphosphoglucuronic acid (UDPGLcUA) in the human fetal liver, kidney and placenta. Eur J Drug Metab Pharmacokinet. 2000;25(3–4):161–3.
56. Kang JH, Kondo F. Determination of bisphenol A in milk and dairy products by high-performance liquid chromatography with fluorescence detection. J Food Prot. 2003;66(8):1439–43.
57. Kawamura Y, et al. Bisphenol A in domestic and imported canned foods in Japan. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2014;31(2):330–40.
58. Cao XL, Corriveau J, Popovic S. Migration of bisphenol A from can coatings to liquid infant formula during storage at room temperature. J Food Prot. 2009;72(12):2571–4.
59. Onn Wong K, Woon Leo L, Leng Seah H. Dietary exposure assessment of infants to bisphenol A from the use of polycarbonate baby milk bottles. Food Addit Contam. 2005;22(3):280–8.
60. Lorber M, et al. Exposure assessment of adult intake of bisphenol A (BPA) with emphasis on canned food dietary exposures. Environ Int. 2015;77:55–62.
61. Bemrah N, et al. Assessment of dietary exposure to bisphenol A in the French population with a special focus on risk characterisation for pregnant French women. Food Chem Toxicol. 2014;72:90–7.
62. Mercogliano R, Santonicola S. Investigation on bisphenol A levels in human milk and dairy supply chain: a review. Food Chem Toxicol. 2018;114:98–107.
63. EFSA. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: executive summary. EFSA J. 2015;13:3978–4599.
64. Lakind JS, Naiman DQ. Bisphenol A (BPA) daily intakes in the United States: estimates from the 2003-2004 NHANES urinary BPA data. J Expo Sci Environ Epidemiol. 2008;18(6):608–15.
65. Lakind JS, Naiman DQ. Daily intake of bisphenol A and potential sources of exposure: 2005-2006 National Health and Nutrition Examination Survey. J Expo Sci Environ Epidemiol. 2011;21(3):272–9.
66. Aris A. Estimation of bisphenol A (BPA) concentrations in pregnant women, fetuses and nonpregnant women in eastern townships of Canada. Reprod Toxicol. 2014;45:8–13.
67. Tan BL, Mohd MA. Analysis of selected pesticides and alkylphenols in human cord blood by gas chromatograph-mass spectrometer. Talanta. 2003;61(3):385–91.
68. Troisi J, et al. Placental concentrations of bisphenol A and birth weight from births in the Southeastern U.S. Placenta. 2014;35(11):947–52.
69. Huo W, et al. Maternal urinary bisphenol A levels and infant low birth weight: a nested case-control study of the Health Baby Cohort in China. Environ Int. 2015;85:96–103.

70. Liu C, et al. Associations between maternal phenolic exposure and cord sex hormones in male newborns. Hum Reprod. 2016;31(3):648–56.
71. Arbuckle TE, et al. Exposure to free and conjugated forms of bisphenol A and triclosan among pregnant women in the MIREC cohort. Environ Health Perspect. 2015;123(4): 277–84.
72. Vandenberg LN, et al. Urinary, circulating, and tissue biomonitoring studies indicate widespread exposure to bisphenol A. Environ Health Perspect. 2010;118(8):1055–70.
73. Bushnik T, et al. Lead and bisphenol A concentrations in the Canadian population. Health Rep. 2010;21(3):7–18.
74. Zhang Z, et al. Urinary bisphenol A concentrations and their implications for human exposure in several Asian countries. Environ Sci Technol. 2011;45(16):7044–50.
75. Becker K, et al. GerES IV: phthalate metabolites and bisphenol A in urine of German children. Int J Hyg Environ Health. 2009;212(6):685–92.
76. Ikezuki Y, et al. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Hum Reprod. 2002;17(11):2839–41.
77. Schonfelder G, et al. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ Health Perspect. 2002;110(11):A703–7.
78. Lee J, et al. Bisphenol A distribution in serum, urine, placenta, breast milk, and umbilical cord serum in a birth panel of mother-neonate pairs. Sci Total Environ. 2018;626: 1494–501.
79. Calafat AM, et al. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. Environ Health Perspect. 2005;113(4):391–5.
80. Casas L, et al. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int. 2011;37(5):858–66.
81. Arbuckle TE, et al. Maternal and infant exposure to environmental phenols as measured in multiple biological matrices. Sci Total Environ. 2015;508:575–84.
82. Lee YJ, et al. Maternal and fetal exposure to bisphenol A in Korea. Reprod Toxicol. 2008;25(4):413–9.
83. Shekhar S, et al. Detection of phenolic endocrine disrupting chemicals (EDCs) from maternal blood plasma and amniotic fluid in Indian population. Gen Comp Endocrinol. 2017;241:100–7.
84. Fernandez MF, et al. Bisphenol A and other phenols in human placenta from children with cryptorchidism or hypospadias. Reprod Toxicol. 2016;59:89–95.
85. Ye X, et al. Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching-high performance liquid chromatography-isotope dilution tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2006;831(1–2):110–5.
86. Yi B, et al. Association between endocrine disrupting phenols in colostrums and maternal and infant health. Int J Endocrinol. 2013;2013:282381.
87. Gerlowski LE, Jain RK. Physiologically based pharmacokinetic modeling: principles and applications. J Pharm Sci. 1983;72(10):1103–27.
88. Karrer C, et al. Physiologically based pharmacokinetic (PBPK) Modeling of the bisphenols BPA, BPS, BPF, and BPAF with new experimental metabolic parameters: comparing the pharmacokinetic behavior of BPA with its substitutes. Environ Health Perspect. 2018;126(7):077002.
89. Shin BS, et al. Physiologically based pharmacokinetics of bisphenol A. J Toxicol Environ Health A. 2004;67(23–24):1971–85.
90. Hotchkiss AK, et al. Fifteen years after "Wingspread"—environmental endocrine disrupters and human and wildlife health: where we are today and where we need to go. Toxicol Sci. 2008;105(2):235–59.
91. McLachlan JA, Simpson E, Martin M. Endocrine disrupters and female reproductive health. Best Pract Res Clin Endocrinol Metab. 2006;20(1):63–75.
92. Xu LC, et al. Evaluation of androgen receptor transcriptional activities of bisphenol A, octylphenol and nonylphenol in vitro. Toxicology. 2005;216(2–3):197–203.

93. Kabuto H, Amakawa M, Shishibori T. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci. 2004;74(24):2931–40.
94. Alizadeh M, et al. Altered allergic cytokine and antibody response in mice treated with bisphenol A. J Med Investig. 2006;53(1–2):70–80.
95. Sawai C, Anderson K, Walser-Kuntz D. Effect of bisphenol A on murine immune function: modulation of interferon-gamma, IgG2a, and disease symptoms in NZB X NZW F1 mice. Environ Health Perspect. 2003;111(16):1883–7.
96. Mathieu-Denoncourt J, et al. Plasticizer endocrine disruption: highlighting developmental and reproductive effects in mammals and non-mammalian aquatic species. Gen Comp Endocrinol. 2015;219:74–88.
97. Krishnan AV, et al. Bisphenol-A: an estrogenic substance is released from polycarbonate flasks during autoclaving. Endocrinology. 1993;132(6):2279–86.
98. McCarthy MM. Estradiol and the developing brain. Physiol Rev. 2008;88(1):91–124.
99. Speroni L, et al. New insights into fetal mammary gland morphogenesis: differential effects of natural and environmental estrogens. Sci Rep. 2017;7:40806.
100. Berger SL, et al. An operational definition of epigenetics. Genes Dev. 2009;23(7):781–3.
101. Zhang Q, et al. Exposure to bisphenol-A affects fear memory and histone acetylation of the hippocampus in adult mice. Horm Behav. 2014;65(2):106–13.
102. Zhang XF, et al. Bisphenol A exposure modifies DNA methylation of imprint genes in mouse fetal germ cells. Mol Biol Rep. 2012;39(9):8621–8.
103. Yaoi T, et al. Genome-wide analysis of epigenomic alterations in fetal mouse forebrain after exposure to low doses of bisphenol A. Biochem Biophys Res Commun. 2008;376(3):563–7.
104. Weng YI, et al. Epigenetic influences of low-dose bisphenol A in primary human breast epithelial cells. Toxicol Appl Pharmacol. 2010;248(2):111–21.
105. Tilghman SL, et al. Endocrine disruptor regulation of microRNA expression in breast carcinoma cells. PLoS One. 2012;7(3):e32754.
106. Anway MD, et al. Epigenetic transgenerational actions of endocrine disruptors and male fertility. Science. 2005;308(5727):1466–9.
107. Anway MD, Skinner MK. Epigenetic transgenerational actions of endocrine disruptors. Endocrinology. 2006;147(6 Suppl):S43–9.
108. Kundakovic M, Champagne FA. Epigenetic perspective on the developmental effects of bisphenol A. Brain Behav Immun. 2011;25(6):1084–93.
109. Calhoun KC, et al. Bisphenol A exposure alters developmental gene expression in the fetal rhesus macaque uterus. PLoS One. 2014;9(1):e85894.
110. Susiarjo M, et al. Bisphenol A exposure disrupts genomic imprinting in the mouse. PLoS Genet. 2013;9(4):e1003401.
111. Villar-Pazos S, et al. Molecular mechanisms involved in the non-monotonic effect of bisphenol-A on ca2+ entry in mouse pancreatic beta-cells. Sci Rep. 2017;7(1):11770.
112. Zhang Y, et al. Non-monotonic dose-response effect of bisphenol A on rare minnow Gobiocypris rarus ovarian development. Chemosphere. 2016;144:304–11.
113. Miyawaki J, et al. Perinatal and postnatal exposure to bisphenol A increases adipose tissue mass and serum cholesterol level in mice. J Atheroscler Thromb. 2007;14(5):245–52.
114. Braun JM, et al. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics. 2011;128(5):873–82.
115. Rochester JR. Bisphenol A and human health: a review of the literature. Reprod Toxicol. 2013;42:132–55.
116. Philippat C, et al. Prenatal exposure to phenols and growth in boys. Epidemiology. 2014;25(5):625–35.
117. Tang R, et al. Associations of prenatal exposure to phenols with birth outcomes. Environ Pollut. 2013;178:115–20.
118. Wolff MS, et al. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect. 2008;116(8):1092–7.

119. Casas M, et al. Exposure to bisphenol A and phthalates during pregnancy and ultrasound measures of fetal growth in the INMA-Sabadell cohort. Environ Health Perspect. 2016;124(4):521–8.
120. Ferguson KK, et al. Repeated measures analysis of associations between urinary bisphenol-A concentrations and biomarkers of inflammation and oxidative stress in pregnancy. Reprod Toxicol. 2016;66:93–8.
121. Lester F, et al. Impact of exposure to phenols during early pregnancy on birth weight in two Canadian cohort studies subject to measurement errors. Environ Int. 2018;120:231–7.
122. Woods MM, et al. Gestational exposure to endocrine disrupting chemicals in relation to infant birth weight: a Bayesian analysis of the HOME Study. Environ Health. 2017;16(1):115.
123. Minatoya M, et al. Cord blood BPA level and child neurodevelopment and behavioral problems: the Hokkaido Study on Environment and Children's Health. Sci Total Environ. 2017;607-608:351–6.
124. Braun JM, et al. Prenatal bisphenol A exposure and early childhood behavior. Environ Health Perspect. 2009;117(12):1945–52.
125. Harley KG, et al. Prenatal and early childhood bisphenol A concentrations and behavior in school-aged children. Environ Res. 2013;126:43–50.
126. Evans SF, et al. Prenatal bisphenol A exposure and maternally reported behavior in boys and girls. Neurotoxicology. 2014;45:91–9.
127. Perera F, et al. Prenatal bisphenol A exposure and child behavior in an inner-city cohort. Environ Health Perspect. 2012;120(8):1190–4.
128. Roen EL, et al. Bisphenol A exposure and behavioral problems among inner city children at 7-9 years of age. Environ Res. 2015;142:739–45.
129. Braun JM, et al. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environ Health Perspect. 2014;122(5):513–20.
130. Miodovnik A, et al. Endocrine disruptors and childhood social impairment. Neurotoxicology. 2011;32(2):261–7.
131. Minatoya M, et al. Prenatal exposure to bisphenol A and phthalates and behavioral problems in children at preschool age: the Hokkaido Study on Environment and Children's Health. Environ Health Prev Med. 2018;23(1):43.
132. Yolton K, et al. Prenatal exposure to bisphenol A and phthalates and infant neurobehavior. Neurotoxicol Teratol. 2011;33(5):558–66.
133. Rochester JR, Bolden AL, Kwiatkowski CF. Prenatal exposure to bisphenol A and hyperactivity in children: a systematic review and meta-analysis. Environ Int. 2018;114:343–56.
134. Hong SB, et al. Bisphenol A in relation to behavior and learning of school-age children. J Child Psychol Psychiatry. 2013;54(8):890–9.
135. Stein TP, et al. Bisphenol A exposure in children with autism spectrum disorders. Autism Res. 2015;8(3):272–83.
136. Perera F, et al. Bisphenol A exposure and symptoms of anxiety and depression among inner city children at 10-12 years of age. Environ Res. 2016;151:195–202.
137. Casas M, et al. Exposure to bisphenol A during pregnancy and child neuropsychological development in the INMA-Sabadell cohort. Environ Res. 2015;142:671–9.
138. Zhou A, et al. Prenatal exposure to bisphenol A and risk of allergic diseases in early life. Pediatr Res. 2017;81(6):851–6.
139. Donohue KM, et al. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. J Allergy Clin Immunol. 2013;131(3):736–42.
140. Spanier AJ, et al. Prenatal exposure to bisphenol A and child wheeze from birth to 3 years of age. Environ Health Perspect. 2012;120(6):916–20.
141. Spanier AJ, et al. Bisphenol A exposure and the development of wheeze and lung function in children through age 5 years. JAMA Pediatr. 2014;168(12):1131–7.
142. Gascon M, et al. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. J Allergy Clin Immunol. 2015;135(2):370–8.

143. Kim KN, et al. Bisphenol A exposure and asthma development in school-age children: a longitudinal study. PLoS One. 2014;9(10):e111383.
144. Wang IJ, Chen CY, Bornehag CG. Bisphenol A exposure may increase the risk of development of atopic disorders in children. Int J Hyg Environ Health. 2016;219(3):311–6.
145. Holt PG, Sly PD. Non-atopic intrinsic asthma and the 'family tree' of chronic respiratory disease syndromes. Clin Exp Allergy. 2009;39(6):807–11.
146. Braun JM, et al. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ Health Perspect. 2012;120(5):739–45.
147. Casas M, et al. Dietary and sociodemographic determinants of bisphenol A urine concentrations in pregnant women and children. Environ Int. 2013;56:10–8.
148. Yamamoto J, et al. Quantifying bisphenol A in maternal and cord whole blood using isotope dilution liquid chromatography/tandem mass spectrometry and maternal characteristics associated with bisphenol A. Chemosphere. 2016;164:25–31.
149. Guenther K, et al. Endocrine disrupting nonylphenols are ubiquitous in food. Environ Sci Technol. 2002;36(8):1676–80.
150. White R, et al. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology. 1994;135(1):175–82.
151. Kovarova J, et al. Alkylphenol ethoxylates and alkylphenols—update information on occurrence, fate and toxicity in aquatic environment. Pol J Vet Sci. 2013;16(4):763–72.
152. Ying GG, Williams B, Kookana R. Environmental fate of alkylphenols and alkylphenol ethoxylates—a review. Environ Int. 2002;28(3):215–26.
153. Inoue K, et al. Migration of 4-nonylphenol from polyvinyl chloride food packaging films into food simulants and foods. Food Addit Contam. 2001;18(2):157–64.
154. Lu J, et al. Anaerobic degradation behavior of nonylphenol polyethoxylates in sludge. Chemosphere. 2008;71(2):345–51.
155. Soares A, et al. Nonylphenol in the environment: a critical review on occurrence, fate, toxicity and treatment in wastewaters. Environ Int. 2008;34(7):1033–49.
156. Soto AM, et al. p-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. Environ Health Perspect. 1991;92:167–73.
157. Thiele B, et al. Contribution to the structural elucidation of 10 isomers of technical p-nonylphenol. Environ Sci Technol. 2004;38(12):3405–11.
158. Russ AS, et al. Synthesis of branched para-nonylphenol isomers: occurrence and quantification in two commercial mixtures. Chemosphere. 2005;60(11):1624–35.
159. U.S. Environmental Protection Agency. Nonylphenol (NP) and Nonylphenol Ethoxylates (NPEs) Action Plan. 2010, [RIN 2070-ZA09] [cited 2018 May 22]. https://www.epa.gov/sites/production/files/2015-09/documents/rin2070-za09_np-npes_action_plan_final_2010-08-09.pdf.
160. Risk and Policy Analysts Limited (RPA). Nonylphenol risk reduction strategy. 1999.
161. Staples CA, et al. Measuring the biodegradability of nonylphenol ether carboxylates, octylphenol ether carboxylates, and nonylphenol. Chemosphere. 1999;38(9):2029–39.
162. Chen HW, et al. Occurrence and assessment of treatment efficiency of nonylphenol, octylphenol and bisphenol-A in drinking water in Taiwan. Sci Total Environ. 2013;449:20–8.
163. Hawrelak M, Bennett E, Metcalfe C. The environmental fate of the primary degradation products of alkylphenol ethoxylate surfactants in recycled paper sludge. Chemosphere. 1999;39(5):745–52.
164. Ekelund R, et al. Bioaccumulation of 4-nonylphenol in marine animals—a re-evaluation. Environ Pollut. 1990;64(2):107–20.
165. Snyder SA, et al. Bioconcentration of nonylphenol in fathead minnows (Pimephales promelas). Chemosphere. 2001;44(8):1697–702.
166. Andersson AM, Skakkebaek NE. Exposure to exogenous estrogens in food: possible impact on human development and health. Eur J Endocrinol. 1999;140(6):477–85.
167. Ferrara F, et al. Alkylphenols and alkylphenol ethoxylates contamination of crustaceans and fishes from the Adriatic Sea (Italy). Chemosphere. 2005;59(8):1145–50.
168. Lu YY, et al. Daily intake of 4-nonylphenol in Taiwanese. Environ Int. 2007;33(7):903–10.

169. Raecker T, et al. Endocrine disrupting nonyl- and octylphenol in infant food in Germany: considerable daily intake of nonylphenol for babies. Chemosphere. 2011;82(11):1533–40.
170. Fernandes AR, Rose M, Charlton C. 4-Nonylphenol (NP) in food-contact materials: analytical methodology and occurrence. Food Addit Contam. 2008;25(3):364–72.
171. Thomson BM, Cressey PJ, Shaw IC. Dietary exposure to xenoestrogens in New Zealand. J Environ Monit. 2003;5(2):229–35.
172. Acir IH, Guenther K. Endocrine-disrupting metabolites of alkylphenol ethoxylates - a critical review of analytical methods, environmental occurrences, toxicity, and regulation. Sci Total Environ. 2018;635:1530–46.
173. de Jager C, Bornman MS, Oosthuizen JM. The effect of p-nonylphenol on the fertility potential of male rats after gestational, lactational and direct exposure. Andrologia. 1999;31(2):107–13.
174. Fan Q, Li W, Shen L. Adverse effects of exposure to p-nonylphenol on reproductive system of young male rats. Zhonghua Yu Fang Yi Xue Za Zhi. 2001;35(5):344–6.
175. Ferguson SA, et al. Maternal and offspring toxicity but few sexually dimorphic behavioral alterations result from nonylphenol exposure. Neurotoxicol Teratol. 2000;22(4):583–91.
176. Harris CA, et al. Nonylphenol affects gonadotropin levels in the pituitary gland and plasma of female rainbow trout. Environ Sci Technol. 2001;35(14):2909–16.
177. Holdway DA, Hefferman J, Smith A. Multigeneration assessment of nonylphenol and endosulfan using a model Australian freshwater fish, Melanotaenia fluviatilis. Environ Toxicol. 2008;23(2):253–62.
178. Jie X, et al. Toxic effect of gestational exposure to nonylphenol on F1 male rats. Birth Defects Res B Dev Reprod Toxicol. 2010;89(5):418–28.
179. LeBlanc GA, Mu X, Rider CV. Embryotoxicity of the alkylphenol degradation product 4-nonylphenol to the crustacean Daphnia magna. Environ Health Perspect. 2000;108(12):1133–8.
180. Nagao T, et al. Reproductive effects of nonylphenol in rats after gavage administration: a two-generation study. Reprod Toxicol. 2001;15(3):293–315.
181. Yokota H, et al. Life-cycle toxicity of 4-nonylphenol to medaka (Oryzias latipes). Environ Toxicol Chem. 2001;20(11):2552–60.
182. Certa H, et al. Toxicokinetics of p-tert-octylphenol in male Wistar rats. Arch Toxicol. 1996;71(1–2):112–22.
183. Pedersen RT, Hill EM. Identification of novel metabolites of the xenoestrogen 4-tert-octylphenol in primary rat hepatocytes. Chem Biol Interact. 2000;128(3):189–209.
184. Coldham NG, et al. Biotransformation, tissue distribution, and persistence of 4-nonylphenol residues in juvenile rainbow trout (Oncorhynchus mykiss). Drug Metab Dispos. 1998;26(4):347–54.
185. Ferreira-Leach AM, Hill EM. Bioconcentration and distribution of 4-tert-octylphenol residues in tissues of the rainbow trout (Oncorhynchus mykiss). Mar Environ Res. 2001;51(1):75–89.
186. Daidoji T, et al. Glucuronidation and excretion of nonylphenol in perfused rat liver. Drug Metab Dispos. 2003;31(8):993–8.
187. Knaak JB, Eldridge JM, Sullivan LJ. Excretion of certain polyethylene glycol ether adducts of nonylphenol by the rat. Toxicol Appl Pharmacol. 1966;9(2):331–40.
188. Doerge DR, et al. Mass spectrometric determination of p-nonylphenol metabolism and disposition following oral administration to Sprague-Dawley rats. Reprod Toxicol. 2002;16(1):45–56.
189. Muller S, Schmid P, Schlatter C. Pharmacokinetic behavior of 4-nonylphenol in humans. Environ Toxicol Pharmacol. 1998;5(4):257–65.
190. Venkatesan AK, Halden RU. National inventory of alkylphenol ethoxylate compounds in U.S. sewage sludges and chemical fate in outdoor soil mesocosms. Environ Pollut. 2013;174:189–93.
191. Lewis SK, Lech JJ. Uptake, disposition, and persistence of nonylphenol from water in rainbow trout (Oncorhynchus mykiss). Xenobiotica. 1996;26(8):813–9.

192. Colin A, et al. Is drinking water a major route of human exposure to alkylphenol and bisphenol contaminants in France? Arch Environ Contam Toxicol. 2014;66(1):86–99.
193. Gu Y, et al. Characteristics of the alkylphenol and bisphenol A distributions in marine organisms and implications for human health: a case study of the East China Sea. Sci Total Environ. 2016;539:460–9.
194. Korsman JC, et al. Modeling bioaccumulation and biomagnification of nonylphenol and its ethoxylates in estuarine-marine food chains. Chemosphere. 2015;138:33–9.
195. Lee CC, et al. Characteristics of nonylphenol and bisphenol A accumulation by fish and implications for ecological and human health. Sci Total Environ. 2015;502:417–25.
196. Fernandes AR, Rose M, Charlton C. 4-Nonylphenol (NP) in food-contact materials: analytical methodology and occurrence. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2008;25(3):364–72.
197. Niu Y, et al. Bisphenol A and nonylphenol in foodstuffs: Chinese dietary exposure from the 2007 total diet study and infant health risk from formulas. Food Chem. 2015;167:320–5.
198. Gyllenhammar I, et al. 4-Nonylphenol and bisphenol A in Swedish food and exposure in Swedish nursing women. Environ Int. 2012;43:21–8.
199. Cacho JI, et al. Determination of alkylphenols and phthalate esters in vegetables and migration studies from their packages by means of stir bar sorptive extraction coupled to gas chromatography-mass spectrometry. J Chromatogr A. 2012;1241:21–7.
200. Lu J, et al. Analysis of bisphenol A, nonylphenol, and natural estrogens in vegetables and fruits using gas chromatography-tandem mass spectrometry. J Agric Food Chem. 2013;61(1):84–9.
201. Ademollo N, et al. Nonylphenol and octylphenol in human breast milk. Environ Int. 2008;34(7):984–7.
202. Huang YF, et al. Nonylphenol in pregnant women and their matching fetuses: placental transfer and potential risks of infants. Environ Res. 2014;134:143–8.
203. Sise S, Uguz C. Nonylphenol in human breast milk in relation to sociodemographic variables, diet, obstetrics histories and lifestyle habits in a Turkish population. Iran J Public Health. 2017;46(4):491–9.
204. Ozaki A, Baba T. Alkylphenol and bisphenol A levels in rubber products. Food Addit Contam. 2003;20(1):92–8.
205. Loyo-Rosales JE, et al. Migration of nonylphenol from plastic containers to water and a milk surrogate. J Agric Food Chem. 2004;52(7):2016–20.
206. Kawamura Y, Ogawa Y, Mutsuga M. Migration of nonylphenol and plasticizers from polyvinyl chloride stretch film into food simulants, rapeseed oil, and foods. Food Sci Nutr. 2017;5(3):390–8.
207. Chen ML, et al. Association between nonylphenol exposure and development of secondary sexual characteristics. Chemosphere. 2009;76(7):927–31.
208. Chang CH, et al. The association between nonylphenols and sexual hormones levels among pregnant women: a cohort study in Taiwan. PLoS One. 2014;9(8):e104245.
209. Park H, Kim K. Urinary levels of 4-nonylphenol and 4-t-octylphenol in a representative sample of the Korean adult population. Int J Environ Res Public Health. 2017;14(8):932.
210. Li LX, et al. Exposure levels of environmental endocrine disruptors in mother-newborn pairs in China and their placental transfer characteristics. PLoS One. 2013;8(5):e62526.
211. Otaka H, Yasuhara A, Morita M. Determination of bisphenol A and 4-nonylphenol in human milk using alkaline digestion and cleanup by solid-phase extraction. Anal Sci. 2003;19(12):1663–6.
212. Chen GW, et al. Alkylphenols in human milk and their relations to dietary habits in central Taiwan. Food Chem Toxicol. 2010;48(7):1939–44.
213. Chen ML, et al. Biomonitoring of alkylphenols exposure for textile and housekeeping workers. Int J Environ Anal Chem. 2005;85(4–5):335–47.
214. Kawaguchi M, et al. Stir bar sorptive extraction with in situ derivatization and thermal desorption-gas chromatography—mass spectrometry for measurement of phenolic xenoestrogens in human urine samples. J Chromatogr B Analyt Technol Biomed Life Sci. 2005;820(1):49–57.

215. Mao L, et al. Determination of environmental estrogens in human urine by high performance liquid chromatography after fluorescent derivatization with p-nitrobenzoyl chloride. Anal Chim Acta. 2004;522:241–6.
216. Balakrishnan B, et al. Passage of 4-nonylphenol across the human placenta. Placenta. 2011;32(10):788–92.
217. Chen ML, et al. Quantification of prenatal exposure and maternal-fetal transfer of nonylphenol. Chemosphere. 2008;73(1 Suppl):S239–45.
218. Laws SC, et al. Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. Toxicol Sci. 2000;54(1):154–67.
219. Routledge EJ, Sumpter JP. Structural features of alkylphenolic chemicals associated with estrogenic activity. J Biol Chem. 1997;272(6):3280–8.
220. Jin S, et al. Enhanced effects by mixtures of three estrogenic compounds at environmentally relevant levels on development of Chinese rare minnow (Gobiocypris rarus). Environ Toxicol Pharmacol. 2012;33(2):277–83.
221. Ishibashi H, et al. Reproductive effects and bioconcentration of 4-nonylphenol in medaka fish (Oryzias latipes). Chemosphere. 2006;65(6):1019–26.
222. Koenig S, et al. Biliary PAH and alkylphenol metabolites, biomarker enzyme activities, and gene expression levels in the deep-sea fish Alepocephalus rostratus. Environ Sci Technol. 2013;47(6):2854–61.
223. Gray MA, Metcalfe CD. Induction of testis-ova in Japanese medaka (Oryzias latipes) exposed to p-nonylphenol. Environ Toxicol Chem. 1997;16:1082–6.
224. Sayed Ael D, Mahmoud UM, Mekkawy IA. Reproductive biomarkers to identify endocrine disruption in Clarias gariepinus exposed to 4-nonylphenol. Ecotoxicol Environ Saf. 2012;78:310–9.
225. Naderi M, et al. Effects of 4-nonylphenol on balance of steroid and thyroid hormones in sexually immature male yellowfin seabream (Acanthopagrus latus). Environ Toxicol. 2014;29:459.
226. Wu JJ, et al. Differential effects of nonylphenol on testosterone secretion in rat Leydig cells. Toxicology. 2010;268(1–2):1–7.
227. Furuta M, et al. Effects of p-nonylphenol and 4-tert-octylphenol on the anterior pituitary functions in adult ovariectomized rats. Neuroendocrinology. 2006;84(1):14–20.
228. Bistakova J, et al. Effects of 4-nonylphenol on the steroidogenesis of human adrenocarcinoma cell line (NCI-H295R). J Environ Sci Health A Tox Hazard Subst Environ Eng. 2017;52(3):221–7.
229. Yu PL, et al. Effects of nonylphenol on the production of progesterone on the rats granulosa cells. J Cell Biochem. 2011;112(9):2627–36.
230. Gu W, et al. Maternal exposure to nonylphenol during pregnancy and lactation induces microglial cell activation and pro-inflammatory cytokine production in offspring hippocampus. Sci Total Environ. 2018;634:525–33.
231. Gu W, et al. Mitogen-activated protein kinase signaling is involved in nonylphenol-induced proinflammatory cytokines secretion by BV2 microglia. J Appl Toxicol. 2018;38(7):958–67.
232. Yu J, et al. Effects of perinatal exposure to nonylphenol on delivery outcomes of pregnant rats and inflammatory hepatic injury in newborn rats. Braz J Med Biol Res. 2016;49(12):e5647.
233. Shaliutina O, et al. The in vitro effect of nonylphenol, propranolol, and diethylstilbestrol on quality parameters and oxidative stress in sterlet (Acipenser ruthenus) spermatozoa. Toxicol In Vitro. 2017;43:9–15.
234. Cilingir Yeltekin A, Oguz AR. Antioxidant responses and DNA damage in primary hepatocytes of Van fish (Alburnus tarichi, Guldenstadt 1814) exposed to nonylphenol or octylphenol. Drug Chem Toxicol. 2018;41:415–23.
235. Magnifico MC, et al. Nonylphenol and octylphenol differently affect cell redox balance by modulating the nitric oxide Signaling. Oxidative Med Cell Longev. 2018;2018:1684827.
236. Park KH. Alteration of hepatic anti-oxidant systems by 4-nonylphenol, a metabolite of alkylphenol polyethoxylate detergents, in Far Eastern catfish Silurus asotus. Environ Health Toxicol. 2015;30:e2015006.

237. Jambor T, et al. In vitro effect of 4-nonylphenol on human chorionic gonadotropin (hCG) stimulated hormone secretion, cell viability and reactive oxygen species generation in mice Leydig cells. Environ Pollut. 2017;222:219–25.
238. Kim H, et al. Comparative toxicological evaluation of nonylphenol and nonylphenol polyethoxylates using human keratinocytes. Drug Chem Toxicol. 2018;41:486–91.
239. Duan P, et al. 4-Nonylphenol induces disruption of spermatogenesis associated with oxidative stress-related apoptosis by targeting p53-Bcl-2/Bax-Fas/FasL signaling. Environ Toxicol. 2017;32(3):739–53.
240. Noorimotlagh Z, et al. The possible DNA damage induced by environmental organic compounds: the case of nonylphenol. Ecotoxicol Environ Saf. 2018;158:171–81.
241. Huang W, et al. Nonylphenol induced apoptosis and autophagy involving the Akt/mTOR pathway in prepubertal Sprague-Dawley male rats in vivo and in vitro. Toxicology. 2016;373:41–53.
242. Tabassum H, et al. Role of melatonin in mitigating nonylphenol-induced toxicity in frontal cortex and hippocampus of rat brain. Neurochem Int. 2017;104:11–26.
243. Jie Y, et al. The effects of gestational and lactational exposure to Nonylphenol on c-jun, and c-fos expression and learning and memory in hippocampus of male F1 rat. Iran J Basic Med Sci. 2017;20(4):386–91.
244. Kazemi S, et al. The correlation between nonylphenol concentration in brain regions and resulting behavioral impairments. Brain Res Bull. 2018;139:190–6.
245. Kawaguchi S, et al. Oral exposure to low-dose of nonylphenol impairs memory performance in Sprague-Dawley rats. J Toxicol Sci. 2015;40(1):43–53.
246. Chang LL, Wun WS, Wang PS. In utero and neonate exposure to nonylphenol develops hyperadrenalism and metabolic syndrome later in life. I. First generation rats (F(1)). Toxicology. 2012;301(1–3):40–9.
247. Zhang HY, et al. Perinatal exposure to 4-nonylphenol affects adipogenesis in first and second generation rats offspring. Toxicol Lett. 2014;225(2):325–32.
248. Yang J, et al. The adverse effects of perinatal exposure to nonylphenol on carbohydrate metabolism in male offspring rats. Int J Environ Health Res. 2017;27(5):368–76.
249. Zhang HY, et al. Perinatal exposure to 4-nonylphenol can affect fatty acid synthesis in the livers of F1 and F2 generation rats. Toxicol Res. 2018;7(2):283–92.
250. Messerlian C, et al. Preconception and prenatal urinary concentrations of phenols and birth size of singleton infants born to mothers and fathers from the Environment and Reproductive Health (EARTH) study. Environ Int. 2018;114:60–8.
251. Etzel TM, et al. Urinary triclosan concentrations during pregnancy and birth outcomes. Environ Res. 2017;156:505–11.
252. Ferguson KK, et al. Environmental phenol associations with ultrasound and delivery measures of fetal growth. Environ Int. 2018;112:243–50.
253. Ouyang F, et al. Maternal urinary triclosan level, gestational diabetes mellitus and birth weight in Chinese women. Sci Total Environ. 2018;626:451–7.
254. Guo J, et al. Associations of prenatal exposure to five chlorophenols with adverse birth outcomes. Environ Pollut. 2016;214:478–84.
255. Aker AM, et al. The associations between prenatal exposure to triclocarban, phenols and parabens with gestational age and birth weight in northern Puerto Rico. Environ Res. 2018;169:41–51.
256. Huo W, et al. Urinary level of triclosan in a population of Chinese pregnant women and its association with birth outcomes. Environ Pollut. 2018;233:872–9.
257. Ding G, et al. Prenatal low-level phenol exposures and birth outcomes in China. Sci Total Environ. 2017;607-608:1400–7.
258. Geer LA, et al. Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York. J Hazard Mater. 2017;323(Pt A):177–83.
259. Wang C, et al. Impacts of prenatal triclosan exposure on fetal reproductive hormones and its potential mechanism. Environ Int. 2018;111:279–86.

260. Berger K, et al. Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels. Environ Res. 2018;165:379–86.
261. Harley KG, et al. Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys. Hum Reprod. 2019;34(1):109–17.
262. Buckley JP, et al. Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's Environmental Health Study. Environ Int. 2016;91:350–6.
263. Kalloo G, et al. Early life triclosan exposure and child adiposity at 8 years of age: a prospective cohort study. Environ Health. 2018;17(1):24.
264. Philippat C, et al. Prenatal exposure to nonpersistent endocrine disruptors and behavior in boys at 3 and 5 years. Environ Health Perspect. 2017;125(9):097014.
265. Nakiwala D, et al. In-utero exposure to phenols and phthalates and the intelligence quotient of boys at 5 years. Environ Health. 2018;17(1):17.
266. Braun JM, et al. Prenatal phthalate, triclosan, and bisphenol A exposures and child visual-spatial abilities. Neurotoxicology. 2017;58:75–83.
267. Etzel T, et al. Prenatal urinary triclosan concentrations and child neurobehavior. Environ Int. 2018;114:152–9.
268. Buckley JP, et al. Associations of prenatal environmental phenol and phthalate biomarkers with respiratory and allergic diseases among children aged 6 and 7years. Environ Int. 2018;115:79–88.
269. Vernet C, et al. In utero exposure to select phenols and phthalates and respiratory health in five-year-old boys: a prospective study. Environ Health Perspect. 2017;125(9):097006.
270. Lee-Sarwar K, et al. Prenatal and early-life triclosan and paraben exposure and allergic outcomes. J Allergy Clin Immunol. 2018;142(1):269–278.e15.
271. Ashley-Martin J, et al. Prenatal triclosan exposure and cord blood immune system biomarkers. Int J Hyg Environ Health. 2016;219(4–5):454–7.

Part IV
Important Aspects of Research for Prevention and Strategy

Chapter 17
Adverse Outcome Pathways for Developmental Toxicity

John M. Rogers

Abstract Adverse Outcome Pathways (AOPs) (frameworks for organizing knowledge about the etiology of an adverse phenotypic outcome) for developmental toxicity are still in their infancy yet represent the culmination of literally centuries of thinking and experimentation. Throughout history people have wondered about the origins of birth defects. It was not until the late nineteenth century that experimental teratology demonstrated that development of embryos could be predictably perturbed by noxious agents, and not until the twentieth century did those experiments include mammalian species. In the mid- to late twentieth century scientists began to design experiments in multiple species to understand the etiology of birth defects. Progress was slow due to limitations in both knowledge of developmental biology and technology. These studies typically took place in individual scientists' laboratories, so lack of collaboration was another impediment. Nevertheless, ideas and experimentation abounded, as in the case of the notorious human teratogen thalidomide, which spurred research producing dozens of hypotheses about mechanisms of teratogenesis and the biology underlying the stark species differences in sensitivity. Developmental biologists began to uncover molecular signaling pathways critical for normal morphogenesis and new approaches to studying them. In the first decade of the twenty-first century, teratologists, developmental biologists, and toxicologists came together first to advocate exploiting new knowledge about evolutionarily conserved signaling pathways to design assays for developmental toxicity done in vitro or in alternative species, and again to advocate for development and application of new approaches including high-throughput batteries of in vitro assays using human materials and robotic instrumentation to speed the accrual of new knowledge. At the same time, rapid advances in computational capabilities spawned in silico models of morphogenesis and powerful cheminformatic approaches. Online databases now provide public access to an ever-expanding volume of data and information, and collaborative tools provide accessible platforms for collective thinking. For AOPs,

J. M. Rogers (✉)
Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, Office of Research and Development, United States Environmental Protection Agency, Research Triangle Park, NC, USA
e-mail: rogers.john@epa.gov

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_17

this includes the AOPwiki (http://aopkb.org/), a public repository for AOPs across the broad field of toxicology that allows for public comments on, and contributions to, AOPs under development. There are at present few AOPs for developmental toxicity in the AOPwiki, but the opportunities are great for this valuable resource. This chapter will briefly recount some historical milestones in teratology and articulate several illustrative examples of research on mechanisms of normal and abnormal development that have served to provide the bricks and mortar from which AOPS for developmental toxicity can be built. AOPs emerging from this knowledge are presented to demonstrate the value of this approach and finally, remaining hurdles to effectively applying the AOP framework to human risk assessments will be discussed.

Keywords Teratology · Mechanism · Embryo · Fetus · Etiology · Retinoids Alcohol

17.1 Introduction

The fields of teratology and developmental toxicology are rapidly changing, taking advantage of progress in our understanding of normal and abnormal development as well as the vast opportunities for data sharing and collaboration offered by public databases and computational tools for mining and analysis of databases, data visualization, cheminformatics, and text mining. New tools and approaches for building in vitro and in silico models of normal and abnormal development have emerged in recent years and offer synergistic approaches to building biologically plausible and defensible predictive risk assessments that will not require vertebrate animals. These opportunities have been broadly recognized over the past two decades, as articulated in seminal publications by the National Research Council [1, 2]. While reaching this lofty goal requires continued progress and may take years to come to fruition, the pace of advancement is accelerating rapidly. A good summary of ongoing advancements and the current state of the field has recently been published [3]. A foundational framework for building confidence in innovative new approaches for toxicity testing and ultimately, human risk assessment is the adverse outcome pathway (AOP) framework. Rooted in the centuries-long pursuit of understanding causes and mechanisms of abnormal development, bringing twenty-first century tools and knowledge to bear on developing AOPs for teratogenesis and developmental toxicity promises to open a new era of rapid advancement of our understanding of, and ability to predict, risk of developmental toxicity in humans.

Following a general introduction to AOPs, this chapter will recount historical approaches to, and progress in, understanding of mechanisms of abnormal development, with a selection of illustrative examples of research that contributed to modern AOPs for abnormal development. AOPs will be presented with emphasis on new

technologies and knowledge that has facilitated their development. Finally, remaining challenges to developing AOPs for developmental toxicity and applying them to human risk assessment will be discussed.

17.2 What Is an Adverse Outcome Pathway?

An adverse outcome pathway (AOP) is a framework for organizing knowledge about the sequence of events leading from the initial interaction of an environmental agent with a living organism (the molecular initiating event, MIE) through multiple biological responses at higher levels of biological organization (key events, KEs) to the expression of an apical adverse outcome at the level of the organism or population [4, 5]. The MIE is a biological event (e.g., receptor agonism or enzyme inhibition) that is chemically agnostic, and thus AOPs are likewise chemically agnostic. That a MIE will result in an apical adverse outcome depends on the magnitude of the initial perturbation and the likelihood of invoking stepwise alterations of subsequent KEs in the AOP. The relationships between successive KEs are termed key event relationships (KERs). While AOPs are most conveniently conceptualized as a linear progression (Fig. 17.1a), it is understood that it will often be the case that there will be multiple overlapping and intersecting pathways, possibly from multiple initiating events, leading to multiple adverse

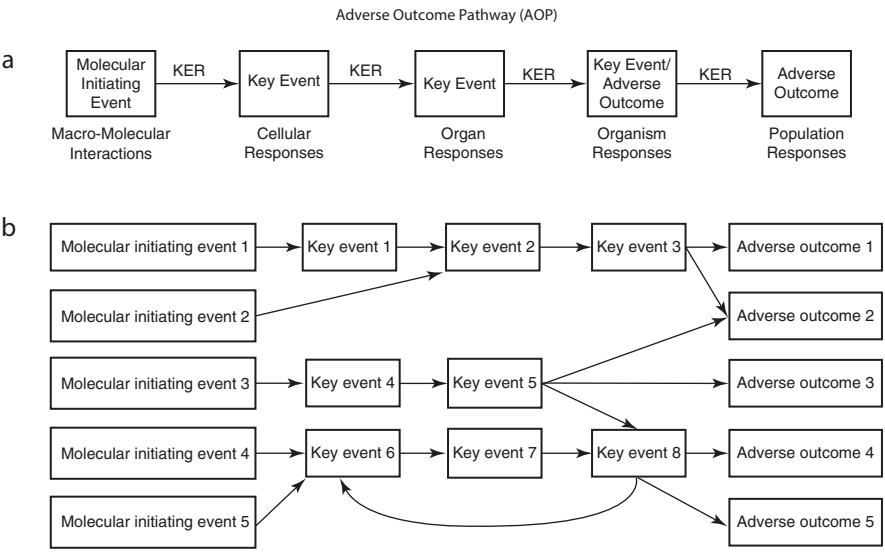


Fig. 17.1 (**a**) Adverse outcome pathway (AOP) basic linear framework showing a molecular initiating event (MIE), key events (KEs), and key event relationships (KERs). (**b**) The more biologically likely situation in which different MIEs can converge on one adverse outcome and/or one MIE can lead to multiple adverse outcomes, creating an AOP network

outcomes (Fig. 17.1b). For chemical risk assessment, AOPs are envisaged to provide knowledge pertaining to extrapolation from current laboratory species to humans (e.g., by comparing KEs and KERs between species, including data from human cells and tissues), development of new alternative models (e.g., biologically plausible in vitro assays or in silico models based on MIEs and KEs), grouping chemicals by mechanism and, potentially, interventions to reduce or prevent an adverse outcome. The general AOP framework and principles for constructing and applying AOPs have been articulated [4–12] and will not be discussed further here. The OECD has established a publicly accessible online repository for AOPs, the AOPWiki, which currently contains 282 toxicity AOPs in various stages of development (October 21, 2019) (http://aopkb.org/). The utility of AOPs is centered in their modular nature that facilitates a living database that expands as research progress provides MIEs, KEs, and KERs for adoption into nascent AOPs. Initial putative AOPs may be only partially defined, lacking an MIE, some KEs, and particularly KERs. Such preliminary AOPs may still be useful to guide experimentation to fill gaps in knowledge. Ultimately, a formal quantitative AOP would have adequate scientific support to identify MIEs and KEs and to mathematically link them through KERS. While AOPs will be increasingly important for risk assessment and as part of integrated approaches to testing and assessment (IATA) [13], development of quantitative AOPs will be very resource intensive; the degree of detail needed in the AOP may vary depending on the problem being addressed. The AOP framework has spawned useful tools like WikiPathways, which links AOP KEs with molecular pathways [14]; the AOP-DB, which relates AOPs to diseases, known chemical-gene interactions, taxonomic information and other biological information gathered from public sources [15]; Effectopedia, an open collaborative platform to display quantitative information on AOPs (https://effectopedia.org); and AOPXplorer, a Cytoscape App for visualization of AOP networks (http://apps.cytoscape.org/apps/aopxplorer). The AOP community of practice will continue to grow as a living, open knowledge collaborative development project at a global scale, which is key to its potential.

17.3 History and Progress in Understanding Birth Defects

Adverse outcome pathways have evolved in part from centuries of thought about the origin of birth defects, so it is useful to start with a brief history of interest in the causes of birth defects and continue to twentieth century experimental approaches to elucidating mechanisms and pathogenesis of teratogenesis. This is the fertile field in which the idea of AOPs for developmental toxicity has grown. Examples below are selected to demonstrate the progression of approaches and knowledge leading to twenty-first century AOPs for developmental toxicity. Much of the knowledge in these AOPs comes from the twentieth century discoveries but the value of the AOP framework will be apparent.

The science of teratology has long sought to understand causes of birth defects. Hippocrates and Aristotle surmised that physical agents could cause birth defects, and ascribed birth defects to adverse maternal impressions and emotions. In 1671, William Harvey articulated the theory of developmental arrest linking birth defects like cleft lip to incomplete development. Experimental teratology in the nineteenth century demonstrated that toxic exposures could cause malformations in chick embryos [16, 17]. The understanding that agents working through specific mechanisms were exploited by the nineteenth and twentieth century embryologists to elucidate mechanisms of normal embryogenesis. In the twentieth century, it became clear from teratology experiments in rats (e.g., [18, 19]) and congenital malformations caused in humans by maternal rubella infection during pregnancy [20] or treatment with the folate-antagonist abortifacient aminopterin [21], that mammalian conceptuses were not necessarily protected from insult in the uterus. The notorious human teratogen, thalidomide, in the late 1950s, caused a spectrum of severe malformations, the best known of which is phocomelia, in which the long bones of the limbs are severely shortened or absent. Alert physicians in Germany and Australia linked this rare malformation to thalidomide prescriptions [22, 23]. James G. Wilson, one of the founders of the Teratology Society, described the genesis of developmental toxicity as the events between the introduction of the causative agent and its final manifestation [24], much the same as the current definition of an AOP. At the time, he listed potential sequential events including molecular mechanisms (now MIEs), cell responses (now KEs), tissue effects (KEs), and outcomes (apical adverse outcome) (Table 17.1). Wilson asserted that scientists could anticipate risks of teratogenic agents by studying the mechanisms by which those agents work. Great strides in our knowledge of mechanisms of normal and abnormal development have since provided a much finer granularity to our understanding of

Table 17.1 Events leading to manifestation of developmental toxicity. Columns are independent lists, rows are not meant to represent sequences of events leading to a specific outcome

Mechanisms	Cell responses	Tissue effects	Outcomes
Mutation	Cell death	Hypoplasia	Impaired growth
Chromosomal breaks	Altered cell replication	Retarded or arrested growth	Tissue/functional deficiency
Altered nucleic acid synthesis	Reduced biosynthesis	Altered differentiation	Malformation
Lack of precursors	Failed cell-cell interactions	Altered morphogenesis	Death
Enzyme inhibition	Impeded morphogenesis	Tissue disruption	
Altered receptor interaction	Altered pattern formation		
Changes in cell membranes	Osmotic imbalance		

Modified from Wilson [24]

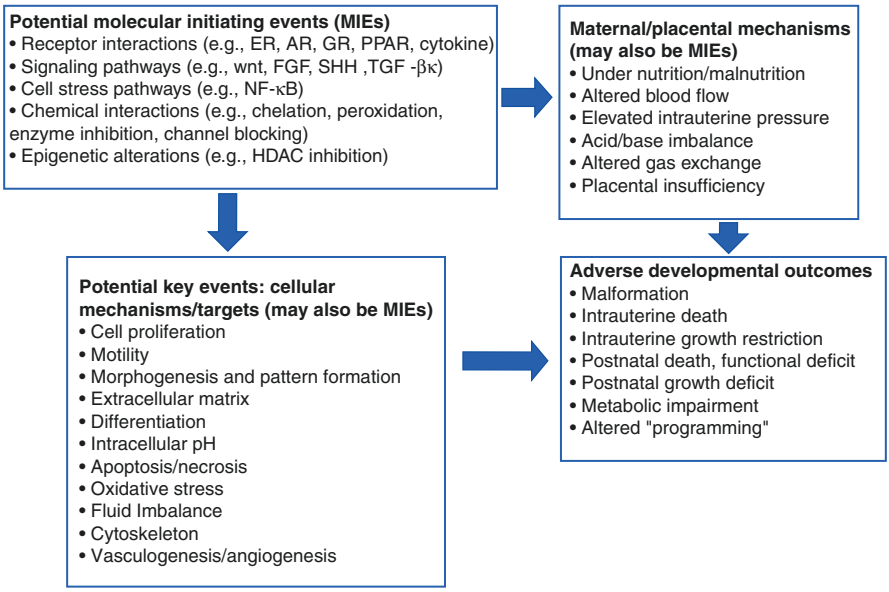


Fig. 17.2 Potential molecular initiating events (MIEs), key events (KEs), and adverse developmental outcomes for adverse outcome pathways (AOPs) for developmental toxicity. AOPs can work through direct effects on the developing organism or indirectly with MIEs and KEs occurring in the mother or the placenta. Modified from [25]. Compared to Table 17.1, published over 50 years earlier, there is overlap as well as new complexity and granularity of understanding

mechanisms and pathogenesis, as evidenced by a twenty-first century update of Wilson's sequence of events (Fig. 17.2) [25]. The conserved biology of development and developmental signaling pathways across phyla offer the opportunity for expansion of both *in vitro* and *in vivo* systems to assess developmental toxicity. The National Research Council (NRC) publication *Scientific Frontiers in Developmental Toxicology and Risk Assessment* (1) articulated these opportunities, indicating at that time that 17 molecular developmental signaling pathways conserved across species and involved in development of multiple structures (Table 17.2) could serve as sentinels for adverse effects on development in in vitro assays and lower organisms as well as mammalian laboratory species. With continued progress in technology and knowledge of toxicology and developmental biology, the NRC published the guiding document *Toxicity Testing in the Twenty-First Century: A Vision and a Strategy* (2) which among other approaches advocated the use of robotics and high-throughput cellular and cell-free assays for toxicity assessment. Current implementations of this vision include the US Environmental Protection Agency's ToxCast program, https://www.epa.gov/chemical-research/toxicity-forecasting) and the Tox21 Consortium, https://ncats.nih.gov/tox21/about/action, which screen thousands of chemicals in hundreds of assays to build predictive models of toxicity, including developmental toxicity.

Table 17.2 The 17 signaling pathways [1]

1. Wingless-Int pathway
2. Transforming growth factor β (receptor serine and threonine kinase) pathway
3. Hedgehog pathway
4. Receptor tyrosine kinase (small G proteins) pathway
5. Notch-delta pathway
6. Cytokine receptor (cytoplasmic tyrosine kinases) pathway (STAT pathway)
7. Interleukin-1-toll nuclear factor-kappa B pathway
8. Nuclear hormone receptor pathway
9. Apoptosis pathway
10. Receptor phosphotyrosine phosphatase pathway
11. Receptor guanylate cyclase pathway
12. Nitric oxide receptor pathway
13. G-protein coupled receptor (large G proteins) pathway
14. Integrin pathway
15. Cadherin pathway
16. Gap junction pathway
17. Ligand-gated cation channel pathway

17.4 Mechanisms and Pathogenesis, Biologically Based Dose-Response Models, and AOPs

The Edema Syndrome and Vascular Disruption In 1958, Grabowski and Paar [26] exposed chicken eggs to hypoxia during different periods between 18 h and 9 days of development. The lowest oxygen levels were embryolethal while less severe hypoxia caused malformations of the eye, face, limbs, and tail. In a later paper [27], Grabowski concluded that an understanding of the underlying mechanisms was necessary. In successive articles published in Science, he showed that lactic acid accumulated in the blood of hypoxic chick embryos and that lactic acid injection produced similar malformations [28], as did injection of albumin or Trypan blue [29]. Hypoxia, albumen and Trypan blue all induced similar ionic changes in the blood of chick embryos. Grabowski recognized that hypoxia-induced malformations were caused by a sequence of events involving edema followed by the formation of clear blisters and hematomas that blocked normal development or disrupted blood supply. He termed this "The Edema Syndrome," [30] (Fig. 17.3). Changes in serum electrolytes after hypoxic exposure suggested impaired osmoregulation [31]. Studies measuring blood pressure in 3- to 5-day old chick embryos revealed a sharp increase in blood pressure prior to the formation of blisters, hematomas, and vascular disruption [32]. Embryos became hypervolemic with distended vasculature that could rupture, resulting in hemorrhage. The Edema Syndrome as conceived by Grabowski in 1964 included an MIE (lactic acid accumulation), a series of KEs and adverse outcomes, with later work adding more KEs (ionic imbalance and elevated blood pressure). Following this

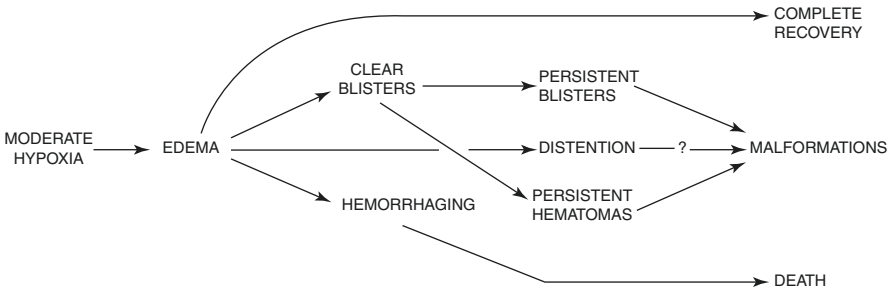


Fig. 17.3 The Edema Syndrome. Based on the effects of hypoxia in chick embryos but greatly expanded by later work, Grabowski [30] described the etiology of malformations in chick embryos that resulted from ionic imbalances leading to edema, blisters, and hemorrhaging that physically interfered with morphogenesis or deprived developing structures of their blood supply

seminal work, several approaches have been used to study hypoxia in pregnant animals, including induced maternal anemia, uterine artery clamping, and vasoconstrictive drugs, and other exposures have been shown to produce similar embryopathy [33]. Results of these studies confirm the basic structure of the Edema Syndrome. Human malformations that comprise deformities of distal structures caused by vascular disruption similar to the Edema Syndrome have been studied by Holmes et al. [34].

Biologically Based Dose-Response (BBDR) Model for 5-Fluorouracil-Induced Hindlimb Defects BBDR models were the forerunners of AOPs. These models use biological and mechanistic information to construct a series of events leading to an apical toxic outcome. The primary difference between BBDR models and AOPs is that BBDR models are designed for a specific toxicant while AOPs are chemical agnostic. In a proof of concept study to design a BBDR model for developmental toxicity [35], the chemotherapeutic drug 5-fluorouracil (5-FU) was chosen because of its well-known biochemical target (inhibition of thymidylate synthetase, TS) and multi-species teratogenicity. Maternal dosing with 5-FU on gestation day 14 in rats caused hindlimb digit defects in a dose-response fashion. Through extensive experimentation, dose-response relationships for TS inhibition, S-phase cell cycle block, early hindlimb bud growth deficits, and fetal digit agenesis were determined. The relationships between these sequential events were mathematically described with Hill-type equations. In AOP parlance, TS inhibition is the MIE, subsequent steps are KEs, the Hill equations are KERs, and digit agenesis is the adverse outcome. Monte Carlo simulations incorporating all the relationships and variability indicated that the BBDR model slightly overpredicted the potency of 5-FU, perhaps because it did not incorporate embryonal repair mechanisms (Fig. 17.4). The BBDR model is useful because it identified a MIE (TS inhibition) that could be assayed in vitro, and that would be expected to be the MIE regardless of the developmental timing of the 5-FU dose, even though different timings of exposure would cause different adverse outcomes.

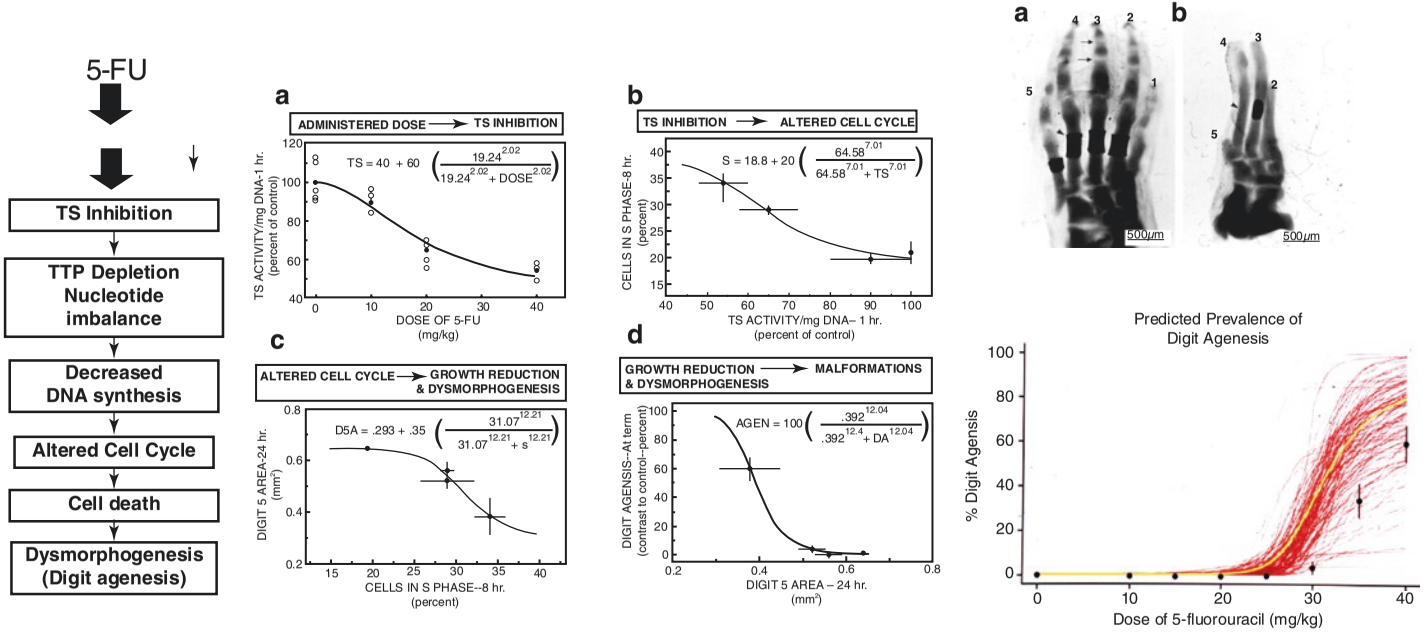


Fig. 17.4 Biologically Based Dose-Response (BBDR) model for hindlimb defects caused by dosing pregnant rats with the chemotherapeutic agent 5-fluorouracil (5-FU). 5-FU is metabolized to 5-FdUMP, an inhibitor of thymidylate synthetase (TS). Inhibition of TS causes nucleotide pool imbalance, decreased DNA synthesis, altered cell cycle, cell death and, ultimately, dysmorphogenesis (left panel). This sequence of events was quantified by measuring TS inhibition, cell cycle blockage in S-phase, limb bud growth inhibition, and digit defects in term fetuses. Here these key events are presented as key event relationships, with (middle panels) (**a**) TS inhibition vs. 5-FU dose; (**b**) S-phase block vs. TS inhibition; (**c**) limb bud digit 5 area vs. S-phase block, and (**d**) Hind limb defects vs. limb bud growth deficit. The lower right panel shows the dose response for hindlimb defects predicted by the sequential quantitative model. The data points and error bars are the experimental data and the curves were produced by a Monte Carlo simulation incorporating measures of variability. The model somewhat overpredicted the adverse outcome, but the experimental data where within the range of the prediction. Control and malformed hindlimb paws from term fetuses are shown in the upper right panel. Adapted from [35]

Thalidomide-Induced Phocomelia and Inhibition of Angiogenesis Given the tragic human experience with thalidomide, the rare limb malformations observed and the species specificity (thalidomide causes phocomelia in humans and nonhuman primates but not rodents), mechanisms underlying the induction of phocomelia have been studied for over 50 years. Stephens [36] identified from the literature 24 proposed mechanisms for thalidomide-induced birth defects; he suspected it was an incomplete list. Putative mechanisms included biochemical alterations involving vitamin B, glutamic acid, acylation, nucleic acids, and oxidative phosphorylation; cellular mechanisms including cell death and cell–cell interactions; and inhibition of nerve and blood vessel outgrowth. More recent hypotheses include effects on angiogenesis [37–41], integrins [42], oxidative DNA damage [43], TNF-α inhibition [44], growth factor antagonism [45, 46], redox imbalance [47, 48], and inhibition of ubiquitin-mediated protein degradation [49, 50]. Parman et al. [43] treated pregnant mice (a resistant species) and rabbits (a sensitive species, although not exhibiting phocomelia) with thalidomide and demonstrated increased DNA oxidation in rabbit but not mouse embryos. Pretreatment with a free-radical scavenging agent ameliorated DNA oxidation and abolished most of the teratogenic effects. Hansen et al. [47, 48] demonstrated a redox shift that reduced binding of the transcription factor NF-κB to its response element in DNA. Target genes for NF-κB include *twist, FGF-10* and *FGF-8,* which are critical for normal limb development. The long history of studying mechanisms of thalidomide teratogenesis and the many partially described mechanisms illustrates the inefficiency of individual efforts to pursue one's favorite hypothesis without a broader framework and collaborative research. The redox hypothesis of Parman et al. [43] and Hansen et al. [47, 48] made good progress and remains viable and the vasculogenesis/angiogenesis inhibition mechanism continues to be pursued. A current hypothesis is that these mechanisms are linked, with tissue hypoxia induced by inhibition of blood vessel formation leading to increased reactive oxygen species and tissue damage [41, 51].

An AOP for developmental toxicity based on inhibition of vasculogenesis and angiogenesis in the embryo has been proposed and contains multiple KEs and adverse outcomes [52] (Fig. 17.5) (AOP43, https://aopwiki.org/aops/43). Phocomelia as produced in humans by thalidomide is one potential adverse outcome, but the rapidly growing embryo is highly dependent on an increasing blood supply such that other malformations, fetal growth deficits, and mortality are also plausible adverse outcomes, dependent on the spatiotemporal occurrence of vascular disruption. A primary KE in this AOP is disruption of vascular endothelial growth factor receptor (VEGFR) signaling, which can lead directly to reduced angiogenic sprouting. Downstream KEs include altered chemokine signaling, extracellular matrix changes, altered hemodynamics, hypoxia, impaired tissue growth, and altered differentiation. Using the US Environmental Protection Agency's ToxCast in vitro battery, a subset of 23 assays representing six target features relevant to vasculogenesis (VEGFR, TIE2, chemokine CCL2 signaling, plasminogen activating system, chemokine CXCL10 upregulation, and urokinase-type plasminogen activator receptor) was used to evaluate 309 ToxCast Phase I chemicals for

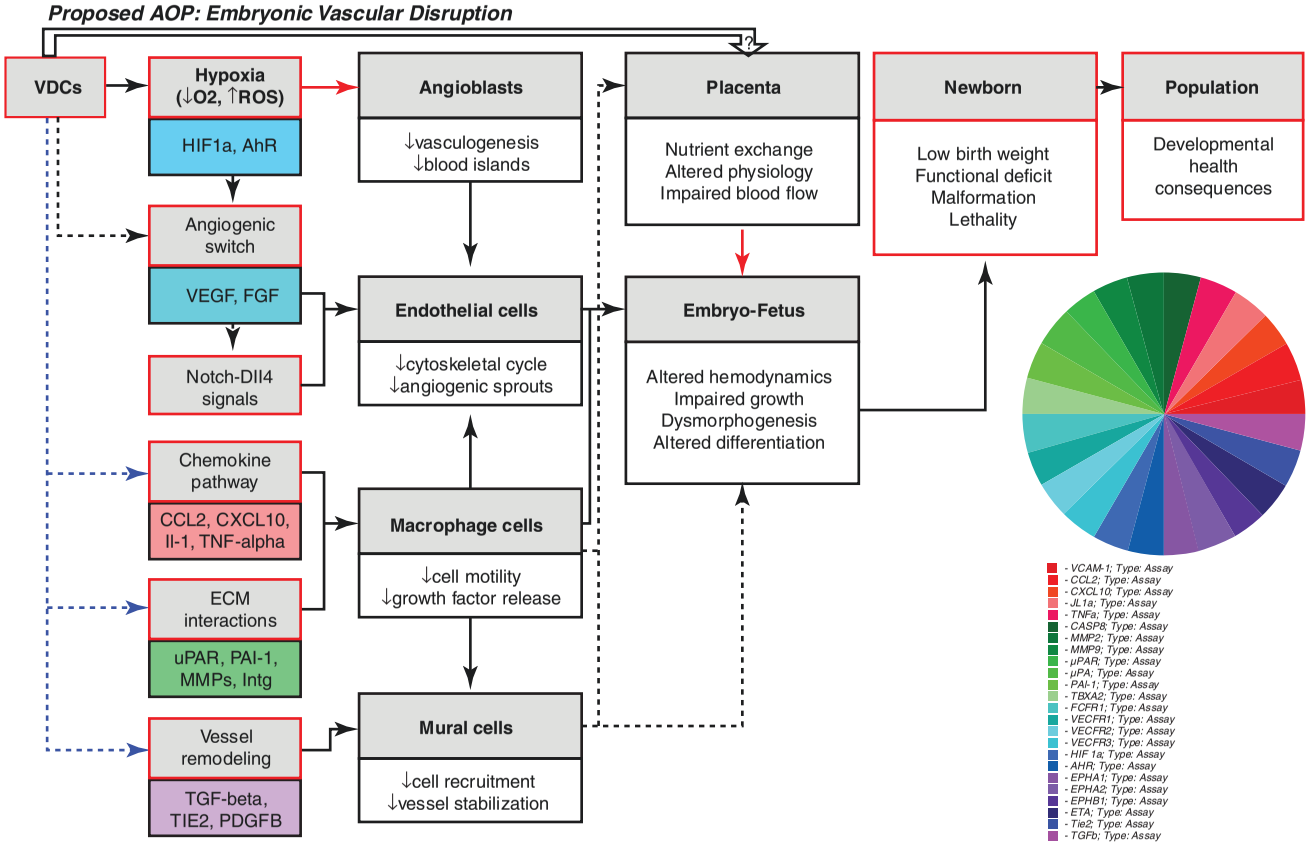


Fig. 17.5 Adverse outcome pathway for inhibition of vasculogenesis/angiogenesis leading to adverse developmental outcomes. Interference with the vascular endothelial growth factor receptor (VEGFR) is one molecular initiating event, but others are shown as well. There are multiple intersecting and interacting key events and pathways in this AOP. In the lower right is a list of ToxCast assays relevant to vascular disruption and events in the AOP that were used to screen chemicals for vascular disrupting potential. Modified from [52, 53]; US government work not subject to copyright

vascular disrupting potential [53]. Thalidomide and its antiangiogenic analog 5HPP-33 were active reference compounds. A scoring system was used to rank the 309 chemicals, and 123 chemicals with a score above the mean were considered potential vascular disrupting chemicals. Many had no previously known potential for vascular disrupting activity, but were known developmental toxicants in vivo. Thalidomide and 5HPP-33 both scored as vascular disruptors. Using the CompuCell3D modeling platform (http://www.compucell3d.org), a computational model based on cellular behaviors and cell signaling pathways was developed to predict disruption of blood vessel development [54]. Molecular targets in the model included some of those chosen for the ToxCast vasculogenesis set of in vitro assays discussed above. A vasculogenesis assay in zebrafish provided a rapid in vivo screen to test chemicals in a whole organism [55]. To examine the effects of vascular disrupting chemicals on mammalian systems, Ellis-Hutchings et al. [56] examined the effects of the antiangiogenic compounds 5-HPP-33 (synthetic thalidomide analog) and TNP-470 (a synthetic fumagillin analog) in rat whole embryo culture. These compounds inhibit angiogenesis through different mechanisms; 5HPP-33 is a microtubule disruptor, while TNP-470 is a METAP II inhibitor. TNP-470 was more potent, causing dysmorphogenesis with an AC50 of 0.038 μM compared to 5HPP-33 which reduced embryo viability with an AC50 of 21.2 μM. These results both confirm the sensitivity of embryogenesis to vascular disruption and demonstrate the variety of adverse outcomes that may result. The antiangiogenic properties of thalidomide and four synthetic phthalimide derivatives were recently compared in a 3D microfluidic model [57]. The analogs were more potent than thalidomide at inhibiting angiogenesis in the system, and the most potent analog was tested in zebrafish alongside thalidomide.

Retinoid-Induced Craniofacial and CNS Malformations All-trans retinoic acid (ATRA) is an endogenous signaling molecule that can bind with its receptors to form transcription factor complexes that activate or repress gene transcription. Many ATRA target genes are essential for embryogenesis and organogenesis and so ATRA is a morphogen that is required at specific times and places; either too much or too little is teratogenic. ATRA imbalance or synthetic retinoids can produce a wide range of malformations. This was nicely illustrated by Shenefelt [59], who administered single doses of ATRA at quarter-day intervals to pregnant golden hamsters. The timing of ATRA treatment determined the malformations induced (Fig. 17.6). Knowledge of ATRA signaling pathways essential for development and sensitive to disruption has accrued over several decades. Excess vitamin A (retinol) was shown to be teratogenic in rats over 60 years ago [60] causing malformations of the face, limbs, heart, CNS, and skeleton. ATRA causes the same malformations in mice [61] and hamsters [59]. The developmental effects of retinol, retinoic acid, and related chemicals that bind to receptors and affect gene expression were intensively studied in the 1990s [62–66]. Targeted gene disruption by homologous recombination, developed in the 1980s [67], was used to study effects of loss-of-function of

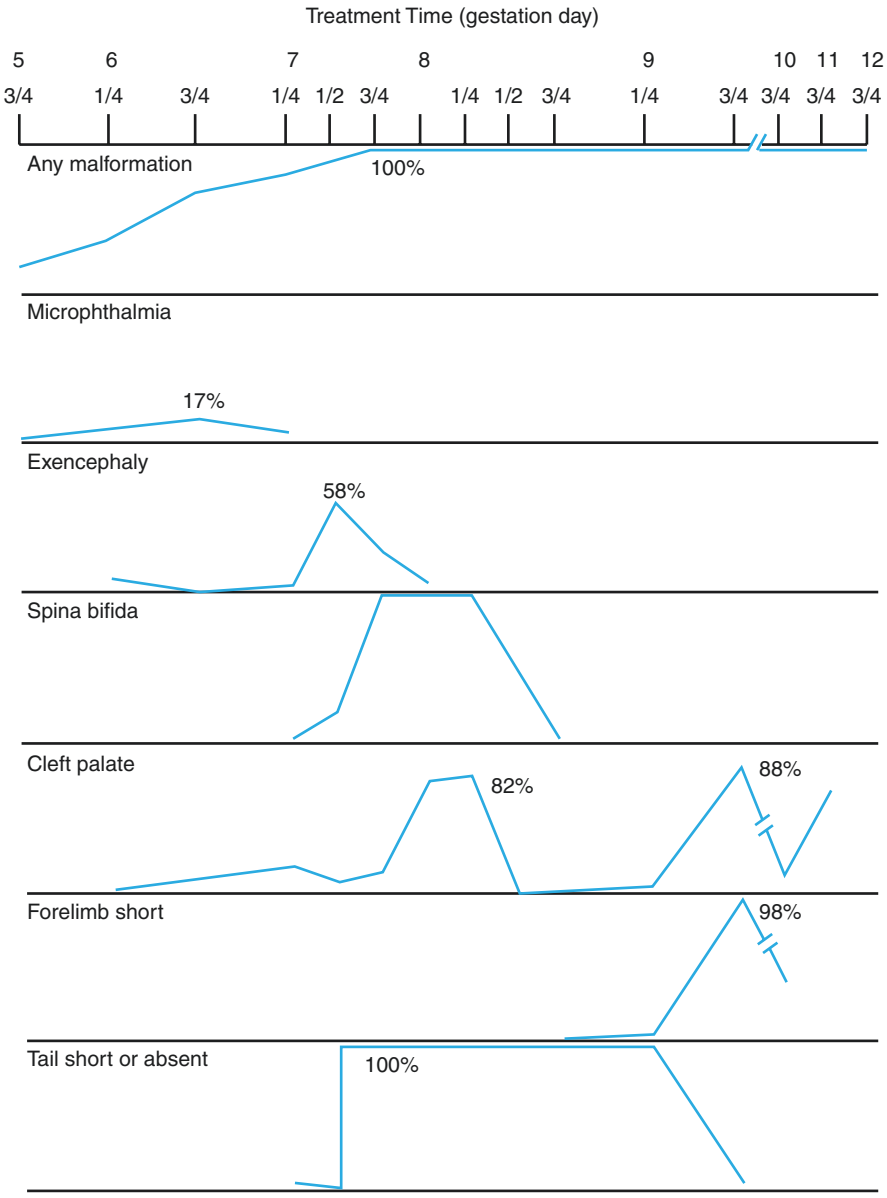


Fig. 17.6 Effects of carefully-timed maternal doses of all-trans retinoic acid (ATRA). Pregnant hamsters were dosed at quarter-day intervals with ATRA and fetuses were evaluated near term. The incidence of specific malformations exhibited an exquisite dependence on time of dose, and the data overall show the broad developmental toxicity of ATRA. Modified and redrawn from [59]

the retinoic acid receptor (RAR) family of transcription factors. Chambon and colleagues deleted genes for multiple RAR forms in mice, producing neonates with malformations of the skeleton, limb, and multiple organs [63, 64]. Gene targeting and transgenic strategies allowed modification of gene expression with temporospatial specificity and gene editing using CRISPR-Cas9 allowed modification of specific gene sequences. Kumar et al. [68] used CRISPR-Cas9 to produce mouse embryos with double nuclear receptor corepressor (*Ncor1* and *Ncor2*) mutants, which exhibited small somites and cardiac distention, a phenotype observed in ATRA-deficient embryos. Retinoic acid repression of *Fgf8* expression is required for morphogenesis, and this study demonstrated that NCOR1 and NCOR2 proteins redundantly mediate repression of *Fgf8* by ATRA. Using in situ hybridization and reporter transgenes, it has been shown that ATRA activates hox genes. Marshall et al. [69] provided the first evidence that some ATRA-induced malformations in mouse embryos are related to changes in hox gene expression.

An AOP for neural tube and axial defects involving ATRA metabolism and signaling demonstrates the value of focusing on signaling pathways rather than adverse apical outcomes [58]. ATRA is an essential morphogen that is a derivative of vitamin A and a teratogen in multiple species including humans [70]. The ATRA signaling pathway is involved in the development of multiple tissues and organs including the CNS, heart, face, lung, and limbs, and excess or deficiency of ATRA during development has the potential to cause malformation of any of these organs. The AOP that these investigators put together listed ATRA imbalance (excess or deficiency) as the MIE, cellular effects on proliferation, migration and differentiation, and tissue patterning effects on the neural tube or embryo axis as KEs, with stage-dependent adverse outcomes including neural tube defects, heart, face, and limb malformations, and cleft palate (Fig. 17.7). Because of the vast literature on ATRA in development, there are many ATRA-regulated genes listed in the AOP, and expression of these genes can be used as biomarkers of perturbation of the ATRA pathway. The authors assert that the retinoid pathway AOP is plausibly related to adverse outcomes caused by multiple developmental toxicants. Prominent among these is the fetal alcohol spectrum disorder caused by ethanol, as described below.

Fetal Alcohol Spectrum Disorder Prenatal ethanol exposure causes the fetal alcohol spectrum disorder (FASD), which in its most severe form manifests as the fetal alcohol syndrome (FAS), first described by Jones and Smith [71, 72]. Alcohol's developmental toxicity has since been documented in hundreds of clinical, epidemiologic, and experimental studies, revealing a wide array of effects [73–76]. The FAS includes craniofacial dysmorphology, growth retardation, and intellectual deficiencies [73, 77, 78]. Animal models include the mouse [79–81], chicken [82], and zebrafish [83]. The molecular mechanisms underlying FAS are not well understood, as alcohol is a promiscuous teratogen with multiple molecular and cellular targets (e.g., [84–86]). Intraperitoneal injection of ethanol to pregnant C57Bl/6J mice during gastrulation [80, 87] has been used to study the etiology and pathogenesis of craniofacial effects seen in FAS. Fetuses of pregnant mice exposed to ethanol in this manner exhibit a phenotype like human FAS, including microcephaly, microphthal-

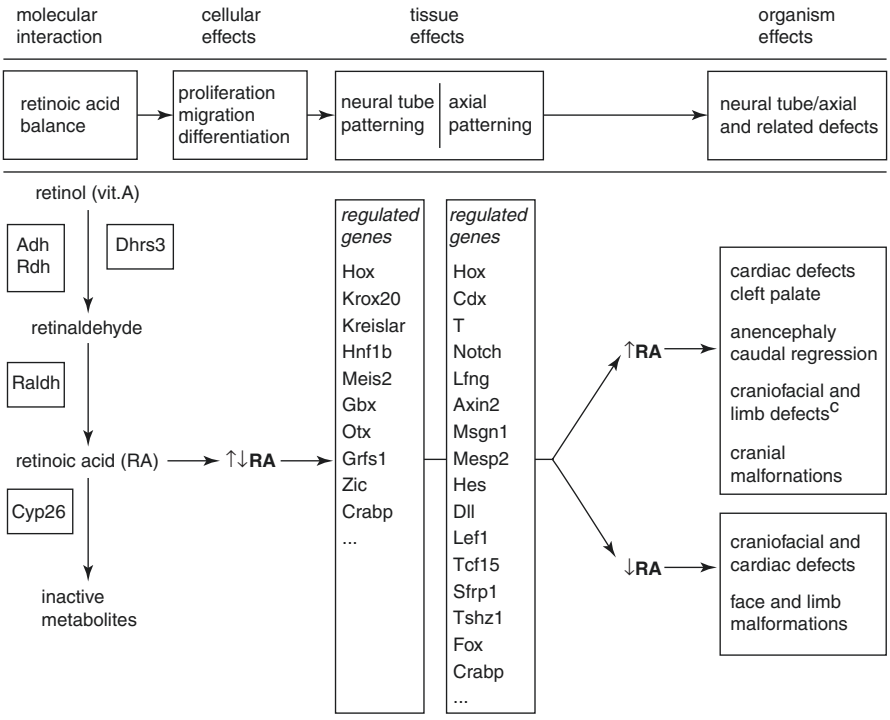


Fig. 17.7 Adverse Outcome Pathway for neural tube and axial malformations by disruption of retinoic acid balance in the embryo. Molecular, cellular, tissue, and organismal effects are illustrated and metabolic enzymes and responsive genes are listed. Either an excess or a deficit of retinoic acid can be teratogenic, so a homeostatic balance is required for normal development. Dependent on timing of retinoic imbalance, various malformations can result (right panels). *RA* All-trans retinoic acid, *Adh* alcohol dehydrogenase, *Rdh* retinol dehydrogenase, *Dhrs3* dehydrogenase reductase 3, *Raldh* retinaldehyde dehydrogenase, *CYP26* retinoic acid hydroxylase

mia, short palpebral fissures, deficient philtrum, and a long upper lip [80]. Increased apoptosis in sensitive cell populations, including neural crest cells, is a common finding [84, 85, 88]. At the molecular level, ethanol can inhibit the oxidation of retinol to ATRA, reducing ATRA concentrations and affecting ATRA signaling pathways critical for craniofacial development, particularly in neural crest cell derived structures [81].

In a review of the etiology of craniofacial dysmorphogenesis caused by ethanol exposure in the chick embryo, Kiecker [89] nicely synthesized current knowledge of neural crest cell signaling pathways and gene expression affected by prenatal ethanol exposure, including ATRA signaling, G protein-coupled receptor (GCPR), and *BMP4/WNT6* pathways as well as oxidative stress and cytoskeletal effects related to the production of neurocristopathies (Fig. 17.8a). Importantly, the specific neurocristopathic phenotype resulting from exposure is exquisitely dependent on timing of ethanol exposure, which during gastrulation causes reduced neural crest

induction, holoprosencephaly and severe craniofacial defects. Later exposure inhibits neural crest migration and survival, resulting in less severe craniofacial effects, and exposure during facial morphogenesis results in milder facial abnormalities (Fig. 17.8b). As a developmental biologist, Kiecker did not refer to his framework as an AOP, but it is an excellent example that clearly demonstrates the benefits of

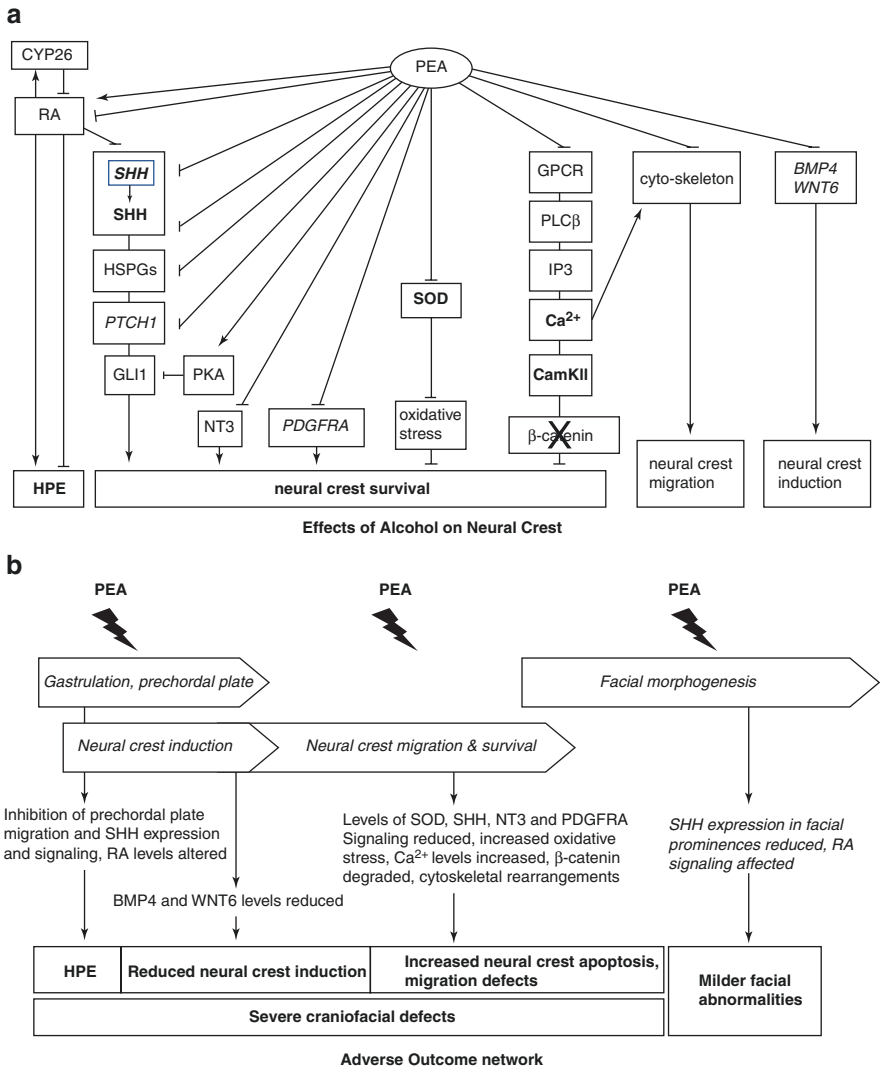


Fig. 17.8 Molecular basis for the effects of prenatal exposure to alcohol (PEA) on neural crest cells and their derivatives in the chick embryo. (**a**) Neural crest cell molecular pathways, genes, and proteins affected by ethanol exposure. (**b**) Spatiotemporal specificity of adverse outcome pathways initiated by ethanol exposure at different times (indicated by lightning bolts) during early development from gastrulation through facial morphogenesis. Modified from [89]

understanding and assessing effects on signaling pathways that are used at multiple places and times during development rather than the time-varying apical outcomes of pathway disruptions.

17.5 Conclusions and Future Directions

Beginning with knowledge of developmental biology accumulated over many decades and continuing with the revolutionary advances in biology and computation over recent years, the ability to probe and understand mechanisms of normal and abnormal development has reached a point that allows a sea change in the way we address developmental toxicity risk assessment. Biotechnological advances like human induced pluripotent stem cells, high-throughput sequencing of the genome, transcriptome and epigenome, metabolomics, gene editing and organotypic culture models, combined with computational power, databases, tools for in silico models of normal and abnormal morphogenesis, cheminformatics, robotics and text mining provide immense opportunities for advancement [3]. Organotypic culture models are complex 3-dimensional in vitro models containing multiple cell types that can recapitulate key aspects of morphogenesis driven by developmental cell signaling pathways. A good example of this is the organoid culture model of morphogenetic fusion of palatal shelves [90–92]. In silico models of morphogenesis using the open-source CompuCell3D platform cited above incorporate signaling pathways into individual cells that interact stochastically using rules that govern their behavior. This platform has been used to build in silico models of vasculogenesis [54], somitogenesis [93, 94], urethral fusion in the genital tubercle [95], and fusion of the secondary palate [96].

Continued progress will require further advancements in our knowledge and technologies. There are a finite number of teratological mechanisms, likely involving signaling pathways that we already understand. Yet, the spatiotemporal patterns of when these pathways are active and how they interact are yet to be completely elucidated. There is much we do not understand about how the single cell zygote uses intrinsic genetic and biochemical information to build an embryo and ultimately a complete organism. An AOP is essentially a normal developmental pathway gone wrong and as such the extant developmental biology literature contains a trove of useful models that can be translated to AOPs. The AOPWiki platform cited above is a great venue for collective thinking about and developing AOPs. Anyone can leave comments or add to the supportive (or not) evidence for AOPs under development, and one need only have a plausible idea for an AOP or part thereof to begin an entry. For adverse developmental outcomes, a great deal of information could be gleaned from the developmental biology literature, and scientists in that field could also benefit from this resource. At present it is greatly underutilized; the VEGFR vascular disruption AOP discussed above (AOP43) is the only mammalian developmental toxicity AOP listed as of this writing. It would be useful to better publicize this resource.

The most difficult part of developing AOPs is understanding key event relationships (KERs), which provide the quantitative aspects of the AOP. KERs will require dose-response relationships between the MIE, KEs and the adverse outcome(s). The BBDR model for 5-FU teratogenicity discussed above provides one way to develop KERs that include measures of uncertainty and can be sequentially applied to estimate quantitatively the adverse outcome. An added difficulty in estimating KERs is that they will certainly be influenced by the physiological milieu in which they occur. Genetics, nutrition, disease, age, repair capacity, and other intrinsic conditions will make KERs somewhat unique to each situation. We know that these factors affect response to toxic exposures, and an effort is made to include these factors in current risk assessments where they are known.

AOPs are useful and may be fully adequate in the absence of defined KERs in some cases, as they qualitatively illuminate key events in the etiology of developmental toxicity, providing clues to the types of chemicals that might trigger the MIE, helping to evaluate human relevance, and facilitating development of in vitro assays. Development of AOPs will be advantaged by systems to organize and integrate the information (data, knowledge) relevant to the task. There are many useful online databases and tools and a vast literature that can be combined to aid in AOP development. Organizing this knowledge involves the creation of a developmental toxicity ontology (a set of concepts and categories to organize knowledge showing properties and relationships between them). One proposal is to base an ontology on presumed mechanism of action for developmental toxicity [25]. In this ontology, molecular information would be linked to traditional toxicological knowledge and human disease states, providing insight about coverage of the universe of toxicity modes of action, and structure for building AOPs.

Scientists have always strived to understand the origin of disease, including congenital malformations. The long slow accumulation of knowledge and techniques has accelerated greatly in recent years, to the extent that it is exceedingly difficult for any scientist to keep up without developing and using tools to gather and organize existing and nascent knowledge. The AOP framework is a great way to do that for toxicology, particularly through crowd-sourcing platforms like the AOPWiki that allows collective thinking and contributions worldwide. Linking AOPs to the many public databases currently available and using the associated tools for visualization and data analysis provides a great deal of synergy. As the AOPwiki platform, and perhaps others, flourish and expand, our ability and necessity to work together will continually increase.

Disclaimer The views expressed herein are those of the author and do not necessarily reflect the views or policies of the United States Environmental Protection Agency.

References

1. National Research Council. Scientific frontiers in developmental toxicology and risk assessment. Washington, DC: The National Academies Press; 2000.

2. National Research Council. Toxicity testing in the 21st century: a vision and a strategy. Washington, DC: The National Academies Press; 2007.
3. Scialli AR, Daston G, Chen C, Coder PS, Euling SY, Foreman J, Hoberman AM, Hui J, Knudsen T, Makris SL, Morford L, Piersma AH, Stanislaus D, Thompson KE. Rethinking developmental toxicity testing: evolution or revolution? Birth Defects Res. 2018;110:840–50.
4. Ankley GT, Bennett RS, Erickson RJ, Hoff DJ, Hornung MW, Johnson RD, Mount DR, Nichols JW, Russom CL, Schmieder PK, Serrano JA, Tietge JE, Villeneuve DL. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem. 2010;29:730–41.
5. OECD. Guidance document on developing and assessing adverse outcome pathways. In: Series on testing and assessment, No. 184, Vol. 6, p. 45. Organization for Economic Cooperation and Development, Environment Directorate, Paris, France. 2013.
6. Vinken M. The adverse outcome pathway concept: a pragmatic tool in toxicology. Toxicology. 2013;312:158–65.
7. Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M, Ottinger MA, Vergauwen L, Whelan M. Adverse outcome pathway (AOP) development I: strategies and principles. Toxicol Sci. 2014;142:312–20.
8. Villeneuve DL, Crump D, Garcia-Reyero N, Hecker M, Hutchinson TH, LaLone CA, Landesmann B, Lettieri T, Munn S, Nepelska M, Ottinger MA, Vergauwen L, Whelan M. Adverse outcome pathway development II: best practices. Toxicol Sci. 2014;142:321–30.
9. Villeneuve DL, Angrish MM, Fortin MC, Katsiadaki I, Leonard M, Margiotta-Casaluci L, Munn S, O'Brien JM, Pollesch NL, Smith LC, Zhang X, Knapen D. Adverse outcome pathway networks II: network analytics. Environ Toxicol Chem. 2018;37:1734–48.
10. Edwards SW, Tan YM, Villeneuve DL, Meek ME, McQueen CA. Adverse outcome pathways-organizing toxicological information to improve decision making. J Pharmacol Exp Ther. 2016;356:170–81.
11. Knapen D, Angrish MM, Fortin MC, Katsiadaki I, Leonard M, Margiotta-Casaluci L, Munn S, O'Brien JM, Pollesch N, Smith LC, Zhang X, Villeneuve DL. Adverse outcome pathway networks I: development and applications. Environ Toxicol Chem. 2018;37:1723–33.
12. Ankley GT, Edwards SW. The adverse outcome pathway: a multifaceted framework supporting 21st century toxicology. Curr Opin Toxicol. 2018;9:1–7.
13. Tollefsen KE, Scholz S, Cronin MT, Edwards SW, de Knecht J, Crofton K, Garcia-Reyero N, Hartung T, Worth A, Patlewicz G. Applying adverse outcome pathways (AOPs) to support integrated approaches to testing and assessment (IATA). Regul Toxicol Pharmacol. 2014;70:629–40.
14. Martens M, Verbruggen T, Nymark P, Grafström R, Burgoon LD, Aladjov H, Torres Andón F, Evelo CT, Willighagen EL. Introducing WikiPathways as a data-source to support adverse outcome pathways for regulatory risk assessment of chemicals and nanomaterials. Front Genet. 2018;9:661.
15. Pittman ME, Edwards SW, Ives C, Mortensen HM. AOP-DB: a database resource for the exploration of adverse outcome pathways through integrated association networks. Toxicol Appl Pharmacol. 2018;343:71–83.
16. Dareste C. Récherches sur la production artificielle des monstruositiés, ou essais de tératogénie expérimentale. Paris: Reinwald; 1877.
17. Dareste C. Récherches sur la production artificielle des monstruositiés, ou essais de tératogénie expérimentale. 2nd ed. Paris: Reinwald; 1891.
18. Warkany J, Nelson RC. Appearance of skeletal abnormalities in the offspring of rats reared on a deficient diet. Science. 1940;92:383–4.
19. Warkany J, Schraffenberger E. Congenital malformations induced in rats by roentgen rays. Am J Roentgenol Radium Ther. 1944;57:455–63.
20. Gregg NM. Congenital cataract following German measles in the mother. Trans Ophthalmol Soc Aust. 1941;3:35–40.
21. Thiersch JB. Therapeutic abortions with a folic acid antagonist 4-aminopteroylglutamic acid administered by the oral route. Am J Obstet Gynecol. 1952;63:1298–304.

22. Lenz W. Kindliche Missbildungen nach Medikament-Einnahme während der Gravidität? Dtsch Med Wochenschr. 1961;86:2555–6.
23. McBride WG. Thalidomide and congenital anomalies. Lancet. 1961;2:1358.
24. Wilson JG. Environment and birth defects. New York: Academic; 1973.
25. Baker N, Boobis A, Burgoon L, Carney E, Currie R, Fritsche E, Knudsen T, Laffont M, Piersma AH, Poole A, Schneider S, Daston G. Building a developmental toxicity ontology. Birth Defects Res. 2018;110:502–18.
26. Grabowski CT, Paar JA. The teratogenic effects of graded doses of hypoxia on the chick embryo. Am J Anat. 1858;103:313–48.
27. Grabowski CT. A quantitative study of the lethal and teratogenic effects of hypoxia on the three-day chick embryo. Am J Anat. 1961;109:25–36.
28. Grabowski CT. Lactic acid accumulation as a cause of hypoxia-induced malformations in the chick embryo. Science. 1961;134:1359–60.
29. Grabowski CT. Teratogenic significance of ionic and fluid imbalances. Science. 1963;142:1064–5.
30. Grabowski CT. The etiology of hypoxia-induced malformations in the chick embryo. J Exp Zool. 1964;157:307–26.
31. Grabowski CT. Physiological changes in the bloodstream of chick embryos exposed to teratogenic doses of hypoxia. Dev Biol. 1966;13:199–213.
32. Grabowski CT, Tsai ET, Toben HR. The effects of teratogenic doses of hypoxia on the blood pressure of chick embryos. Teratology. 1969;2:67–76.
33. Webster WS, Abela D. The effect of hypoxia in development. Birth Defects Res. 2007;81:215–28.
34. Holmes LB, Westgate MN, Nasri H, Toufaily MH. Malformations attributed to the process of vascular disruption. Birth Defects Res. 2018;110:98–107.
35. Shuey DL, Lau C, Logsdon TR, et al. Biologically based dose–response modeling in developmental toxicology: biochemical and cellular sequelae of 5-fluorouracil exposure in the developing rat. Toxicol Appl Pharmacol. 1994;126:129–44.
36. Stephens T. Proposed mechanisms of action in thalidomide embryopathy. Teratology. 1988;38:229–39.
37. D'Amato RJ, Loughman MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci. 1994;91:4082–5.
38. Joussen AM, Germann T, Kirchhof B. Effect of thalidomide and structurally related compounds on cornela angiogenesis is comparable to their teratological potency. Graefes Arch Clin Exp Ophthalmol. 1999;237:952–61.
39. Sauer H, Gunther J, Hescheler J, Wartenberg M. Thalidomide inhibits angiogenesis in embryoid bodies by the generation of hydroxyl radicals. Am J Pathol. 2000;56:151–8.
40. Stephens TD, Bunde CJ, Fillmore BJ. Mechanism of action in thalidomide teratogenesis. Biochem Pharmacol. 2000;59:1489–99.
41. Therapontos C, Erskine L, Gardner EER, Figg WD, Vargesson N. Thalidomide induces limb defects by preventing angiogenic outgrowth during early limb formation. Proc Natl Acad Sci U S A. 2009;106:8573–8.
42. Neubert R, Hinz N, Thiel R, Neubert D. Down-regulation of adhesion receptors on cells of primate embryos as a probable mechanism of the teratogenic action of thalidomide. Life Sci. 1996;58:295–316.
43. Parman T, Wiley MJ, Wells PG. Free radical-mediated oxidative DNA damage in the mechanism of thalidomide teratogenicity. Nat Med. 1999;5:582–5.
44. Argiles JM, Carbo N, Lopez-Soriano FJ. Was tumour necrosis factor-alpha responsible for the fetal malformations associated with thalidomide in the early 1960's? Med Hypotheses. 1998;50:313–8.
45. Stephens TD, Bunde CJW, Torres RD, et al. Thalidomide inhibits limb development through its antagonism of IFG-I+FGF-2+heparin. Teratology. 1998;57:112.
46. Stephens TD, Fillmore BJ. Thalidomide embryopathy: proposed mechanism of action. Teratology. 2000;61:189–95.

47. Hansen JM, Harris KK, Philbert MA, Harris C. Thalidomide modulates nuclear redox status and preferentially depletes glutathione in rabbit limb versus rat limb. J Pharmacol Exp Ther. 2002;300:768–76.
48. Hansen JM, Harris C. A novel hypothesis for thalidomide-induced limb teratogenesis: redox misregulation of the NF-kappaB pathway. Antioxid Redox Signal. 2004;6:1–14.
49. Ito T, Ando H, Handa H. Teratogenic effects of thalidomide: molecular mechanisms. Cell Mol Life Sci. 2011;68:1569–79.
50. Ito T, Handa H. Deciphering the mystery of thalidomide teratogenicity. Congenit Anom. 2012;52:1–7.
51. Vargesson N. Thalidomide-induced teratogenesis: history and mechanisms. Birth Defects Res. 2015;105:140–56.
52. Knudsen TB, Kleinstreuer NC. Disruption of embryonic vascular development in predictive toxicology. Birth Defects Res. 2011;93:312–23.
53. Kleinstreuer NC, Judson RS, Reif DM, et al. Environmental impact on vascular development predicted by high-throughput screening. Environ Health Perspect. 2011;119:1596–603.
54. Kleinstreuer N, Dix D, Rountree M, Baker N, Sipes N, Reif D, Spencer R, Knudsen T. A computational model predicting disruption of blood vessel development. PLoS Comput Biol. 2013;9:e1002996.
55. Tal T, Kilty C, Smith A, LaLone C, Kennedy B, Tennant A, McCollum CW, Bondesson M, Knudsen T, Padilla S, Kleinstreuer N. Screening for angiogenic inhibitors in zebrafish to evaluate a predictive model for developmental vascular toxicity. Reprod Toxicol. 2017;70:70–81.
56. Ellis-Hutchings RG, Settivari RS, McCoy AT, Kleinstreuer N, Franzosa J, Knudsen TB, Carney EW. Embryonic vascular disruption adverse outcomes: linking high throughput signaling signatures with functional consequences. Reprod Toxicol. 2017;70:82–96.
57. Mercurio A, Sharples L, Corbo F, Franchini C, Vacca A, Catalano A, Carocci A, Kamm RD, Pavesi A, Adriani G. Phthalimide derivative shows anti-angiogenic activity in a 3D microfluidic model and no teratogenicity in zebrafish embryos. Front Pharmacol. 2019;10:349.
58. Tonk EC, Pennings JL, Piersma AH. An adverse outcome pathway framework for neural tube and axial defects mediated by modulation of retinoic acid homeostasis. Reprod Toxicol. 2015;55:104–13.
59. Shenefelt RE. Morphogenesis of malformations in hamsters caused by retinoic acid: relation to dose and stage of treatment. Teratology. 1972;5:103–18.
60. Cohlan SQ. Congenital anomalies in the rat produced by excessive intake of vitamin A during pregnancy. Pediatrics. 1954;13:556–67.
61. Kochhar DM. Teratogenic activity of retinoic acid. Acta Pathol Microbiol Scand. 1967;70:398–404.
62. Chambon P. The retinoid signaling pathway: molecular and genetic analyses. Semin Cell Biol. 1994;5:115–25.
63. Lohnes D, Mark M, Mendelsohn C, et al. Function of the retinoic acid receptors (RARs) during development: I. Craniofacial and skeletal abnormalities in RAR double mutants. Development. 1994;120:2723–48.
64. Mendelsohn C, Lohnes D, Décimo D, et al. Function of the retinoic acid receptors (RARs) during development: II. Multiple abnormalities at various stages of organogenesis in RAR double mutants. Development. 1994;120:2749–71.
65. Collins MD, Mao GE. Teratology of retinoids. Annu Rev Pharmacol Toxicol. 1999;39:399–430.
66. Arafa HM, Elmazar MM, Hamada FM, Reichert U, Shroot B, Nau H. Selective agonists of retinoic acid receptors: comparative toxicokinetics and embryonic exposure. Arch Toxicol. 2000;73:547–56.
67. Capecchi MR. Altering the genome by homologous recombination. Science. 1989; 244:1288–92.
68. Kumar S, Cunningham TJ, Duester G. Nuclear receptor corepressors Ncor1 and Ncor2 (Smrt) are required for retinoic acid-dependent repression of Fgf8 during somitogenesis. Dev Biol. 2016;418:204–15.
69. Marshall H, Nonchev S, Sham MH, et al. Retinoic acid alters hindbrain Hox code and induces transformation of rhombomeres 2/3 into a 4/5 identity. Nature. 1992;360:737–41.

70. Piersma AH, Hessel EV, Staal YC. Retinoic acid in developmental toxicology: teratogen, morphogen and biomarker. Reprod Toxicol. 2017;72:53–61.
71. Jones KL, Smith DW. Recognition of the fetal alcohol syndrome in early infancy. Lancet. 1973;2:999–1001.
72. Jones KL, Smith DW, Ulleland CN, Streissguth AP. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet. 1973;1:1267–71.
73. Jones KL. The effects of alcohol on fetal development. Birth Defects Res. 2011;93:3–11.
74. McQuire C, Paranjothy S, Hurt L, et al. Objective measures of prenatal alcohol exposure: a systematic review. Pediatrics. 2016;138:e20160517.
75. Hoyme HE, Kalberg WO, Elliott AJ, et al. Updated clinical guidelines for diagnosing fetal alcohol spectrum disorders. Pediatrics. 2016;138:e20154256.
76. Lunde ER, Washburn SE, Golding MC, et al. Alcohol-induced developmental origins of adult-onset diseases. Alcohol Clin Exp Res. 2016;40:1403–14.
77. Abel EL. Consumption of alcohol during pregnancy: a review of effects on growth and development of offspring. Hum Biol. 1982;54:421–53.
78. Abel EL. Fetal alcohol syndrome: a cautionary note. Curr Pharm Des. 2006;12:1521–9.
79. Chernoff GF. The fetal alcohol syndrome in mice: an animal model. Teratology. 1977;15:223–30.
80. Sulik KK, Johnston MC, Webb MA. Fetal alcohol syndrome: embryogenesis in a mouse model. Science. 1981;214:936–8.
81. Petrelli B, Weinberg J, Hicks GG. Effects of prenatal alcohol exposure (PAE): insights into FASD using mouse models of PAE. Biochem Cell Biol. 2018;96:131–47.
82. Flentke GR, Smith SM. The avian embryo as a model for fetal alcohol spectrum disorder. Biochem Cell Biol. 2018;96:98–106.
83. Fernandes Y, Buckley DM, Eberhart JK. Diving into the world of alcohol teratogenesis: a review of zebrafish models of fetal alcohol spectrum disorder. Biochem Cell Biol. 2018;96:88–97.
84. Sulik KK. Genesis of alcohol-induced craniofacial dysmorphism. Exp Biol Med. 2005;230:366–75.
85. Smith SM. Alcohol and cell death. In: McQueen CA, Daston GP, Knudsen TB, editors. Comprehensive toxicology, Developmental toxicology, vol. 12. 2nd ed. New York: Elsevier; 2010. p. 223–38.
86. Zeisel SH. What choline metabolism can tell us about the underlying mechanisms of fetal alcohol spectrum disorders. Mol Neurobiol. 2011;44:185–91.
87. Sulik KK, Johnston MC. Sequence of developmental alterations following acute ethanol exposure in mice: craniofacial features of the fetal alcohol syndrome. Am J Anat. 1983;166:257–69.
88. Kotch LE, Sulik KK. Experimental fetal alcohol syndrome: proposed pathogenic basis for a variety of associated facial and brain anomalies. Am J Med Genet. 1992;44:168–76.
89. Kiecker C. The chick embryo as a model for the effects of prenatal exposure to alcohol on craniofacial development. Dev Biol. 2016;415:314–25.
90. Belair DG, Wolf CJ, Wood C, Ren H, Grindstaff R, Padgett W, Swank A, MacMillan D, Fisher A, Winnik W, Abbott BD. Engineering human cell spheroids to model embryonic tissue fusion in vitro. PLoS One. 2017;12:e0184155.
91. Belair DG, Wolf CJ, Moorefield SD, Wood C, Becker C, Abbott BD. A three-dimensional organoid culture model to assess the influence of chemicals on morphogenetic fusion. Toxicol Sci. 2018;166:394–408.
92. Wolf CJ, Belair DG, Becker CM, Das KP, Schmid JE, Abbott BD. Development of an organotypic stem cell model for the study of human embryonic palatal fusion. Birth Defects Res. 2018;110:1322–34.
93. Dias AS, de Almeida I, Belmonte JM, Glazier JA, Stern CD. Somites without a clock. Science. 2014;343:791–5.
94. Hester SD, Belmonte JM, Gens JS, Clendenon SG, Glazier JA. A multi-cell, multi-scale model of vertebrate segmentation and somite formation. PLoS Comput Biol. 2011;7:e1002155.
95. Leung MC, Hutson MS, Seifert AW, Spencer RM, Knudsen TB. Computational modeling and simulation of genital tubercle development. Reprod Toxicol. 2016;64:151–61.
96. Hutson MS, Leung MCK, Baker NC, Spencer RM, Knudsen TB. Computational model of secondary palate fusion and disruption. Chem Res Toxicol. 2017;30:965–79.

Chapter 18
Exposomics: The Exposome in Early Life

Léa Maitre and Martine Vrijheid

Abstract Individuals are exposed to a wide range of environmental factors of different nature, social, physical, and chemical over their lifetime. The cumulative effect of these environmental stressors, their interaction with genetic factors and key susceptible developmental stages determines disease risks. The concept of the "exposome"—representing all non-genetic exposures experienced during the life course—was a call to complement the impressive advances made in measuring the human genome with similar technology investment in measuring the environmental component of disease aetiology (Wild, Cancer Epidemiol Biomark Prev 14:1847–1850, 2005). While measuring the exposome is recognized to be extremely challenging due to its dynamic, heterogeneous, and still unknown nature, advances in new and emerging technologies were seen as opportunities to characterize internal and external domains of the exposome in a more holistic way. More than a decade after the exposome concept was first proposed, several projects across Europe and the USA have started implementing at least part of it. This chapter will describe the utility of the exposome concept, its characteristics, how it can be feasibly measured, and its first implementation in health studies, focusing on the early-life periods. Finally, the challenges and future perspectives of exposome research will be presented.

Keywords Exposome · Metabolomics · Exposure sciences · Environmental epidemiology

L. Maitre (✉) · M. Vrijheid
ISGlobal, Barcelona, Spain

Universitat Pompeu Fabra (UPF), Barcelona, Spain

CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
e-mail: lea.maitre@isglobal.org

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_18

18.1 The Utility of the Exposome Research Framework

In encompassing the totality of our environmental exposures, the exposome's main advantage over traditional one-exposure-one-health outcome approaches is that it provides an unprecedented conceptual framework for the study of multiple environmental hazards and their combined effects [1]. As such, the exposome provides input into priority setting and into a wide range of policy issues covering more than one exposure at once. Environmental health policy areas that will benefit most from an exposome approach include any that deal with priority setting and thus require a systematic approach to many suspected environmental risk factors, as well as policy areas that tackle more than one risk factor or pollutant at the time and thus require knowledge on how such factors act together to influence health: from chemical regulations and strategies (e.g., on endocrine disruptors, chemical mixtures, pesticides, food contact materials, cosmetics, air quality), to those tackling urban environments and disease-specific prevention policies. It is increasingly clear that approaches that do not examine complex multifactor effects seem to be ineffective in explaining, let alone preventing the onset of most common non-communicable diseases. Here it is important to recognize the interplay of multiple exposures and the complex "system" in which efforts to reduce the harmful exposome are made (encompassing individuals, communities, organizations, the natural and built environmental, and economic and political forces) [2].

As an integral part of the exposome, internal, biological responses to exposures can be measured at the molecular level using high-throughput omics techniques, which have great potential for broad and powerful characterization of complete sets of biological molecules: metabolomics, proteomics, transcriptomics, and epigenomics. Of particular interest is the identification of biological responses and pathways that respond to and interact with the exposures, leading to adverse health, i.e., "early pathway perturbations". Such information may be used to improve biological plausibility of associations, to understand how different exposures may act on common pathways, and, ultimately, to predict environmental health related disease. Similar to developments in the fields of toxicology and pharmacology, the identification of perturbed pathways of well characterized exposures may allow predicting the public health burden of more recent, less characterized exposures.

Human exposure to environmental hazards is not fixed and changes throughout life stages. The exposome cannot be measured over the whole life course though, and its complexities make it necessary to focus this work on a specific population or part of the life course. At the same time, lifetime health trajectories contain a so-called build-up stage, from conception and early intra-uterine life to late adolescence, which is characterized by rapid successions of developmentally and socially sensitive periods that strongly determine subsequent later disease and ageing trajectories and thereby influence the maximum attained level of health [3, 4]. Starting prevention in early life is a particularly efficient way to shift or improve these trajectories. Within this period, the first 1000 days of life (from preconception until 2 years of age) are often promoted as a window of particular opportunity for

interventions to prevent a range of later diseases, from obesity to neurodegeneration [5]. The exposome is particularly useful in measuring exposures during vulnerable periods that may have pronounced effects at the molecular level but may remain clinically undetectable until adulthood. Children may also display differences in susceptibility to their environment and in the era of personalized medicine, a personal exposome assessment should consider molecular susceptibility. For example, the toxicity of arsenic, a ubiquitous metal whose exposure occurs mainly through the consumption of fish and crustaceans, will depend heavily on the capacity of the liver and potentially gut microbiota to methylate arsenic species [6, 7].

18.2 Characterizing the Exposome

The exposome has been delineated by Wild to include three overlapping and complementary domains based on how each part can be feasibly measured [8], (1) a *personal or "individual" exposome*, which is evaluated on an individual basis, with factors such as specific chemical contaminants (air pollutants, endocrine disruptors, heavy metals, pesticides), physical environmental hazards (noise, temperature, light, UV), behavioural and lifestyle factors (diet, physical activity, sleep, screen time), social and psychosocial factors (stress, social contacts and participation, affluence), medical events such as infections, and their combinations and interactions; (2) a *general "external" exposome* is measured at the macro or neighbourhood level, such as the urban, built, and transport environment, natural and green spaces, climate factors, and the social environment but also at a global level such as governmental policies and immigration patterns; and (3) a third domain assembling *biological responses*, also referred to as the "internal exposome", including all internal processes that respond to and interact with the exposures. It includes epigenetic marks, gene expression, inflammation, metabolism, and the gut microflora. Table 18.1 presents the potential exposures that can be measured for each exposome domain and examples of data sources. We note that some exposures, for example, air pollution, cannot easily be assigned to one domain as they can be measured both at the more specific, individual level and at the external general external level.

18.2.1 The Personal Exposome

This domain of the exposome largely contains exposures measured at the individual level, through a range of exposure assessment methods such as biomonitoring, sensors, geospatial modelling combined with GPS data, and questionnaires, detailed in Table 18.1. Two conceptual approaches have been developed to assess the chemical part of the personal exposome. One approach is from the "bottom-up" using targeted methods to analyse biomarkers in biological samples of selected known

Table 18.1 Concept framework of the "exposome": multiple levels of potential environmental exposures

Domains and levels of measurement	Sublevels	Potential exposures	Potential data sources
The personal exposome Individual	Chemical (biomarker)	– Known toxic chemicals (e.g., lead, flame retardants, pesticides) that can be measured in biological samples indicative of internal exposure	International database: information platform for chemical monitoring (IPCheM), National public health agencies such as Canadian Health Measures Survey (CHMS), US Centers for Disease Control and Prevention (CDC), National Health and Nutrition Examination Survey (NHANES), EU population-based research projects: DEMOCOPHES, HBM4EU or HELIX[a]
	Chemical (unknown)	– Untargeted screening through high-resolution mass spectrometry (HR-MS) platforms in biological samples	
	Chemical (ambient)	– Outdoor air pollution (traffic) – Indoor air pollution (e.g., solid fuel use) – Water sources and consumption patterns	European Environment Agency (EPA) air monitoring network, (e.g., U.S. Census Bureau), population-based survey (e.g., CDC's behavioural risk factor surveillance system), remote sensing, mobile app Existing research projects such as ESCAPE for air pollution, HIWATE for water quality
	Medical events	– Medical history – Injuries – Infections	Physical examinations, participant self-report, health registries, electronic medical records
	Physical	– Ambient UV radiation levels – Household allergens (e.g., pet, mould, etc.) – Light, night time exposure and home light – Road traffic noise	Participant self-report, official sources of data (e.g., U.S. Census Bureau) population-based survey (e.g., CDC's behavioural risk factor surveillance system), remote sensing
	Behavioural/ lifestyle exposures	– Lifestyle, health behaviour (i.e., physical activity, diet, sleep) – Commuting routes and mobility – Time spent in different environments – Mobile technology use/screen time	Participant self-report, mobile app, wrist watches, population-based cohorts
	Social/ psychosocial	– Socio-demographic factors such as age, education level of parents, ethnicity, poverty – Family affluence score, social contact, social participation, house crowding – Psychosocial (e.g., stress)	Participant self-report, mobile app, hair cortisol (for stress), population-based cohorts

General external exposome Local, community	Climate	Temperature, relative humidity, pressure, wind	Daily average from city monitoring stations, climate models
	Built environment	– Population and building density – Street connectivity – Facility density – Walkability – Access to schools, health services – Streetscape, urban roadway design and conditions	Open source data such as OpenStreetMap, Private GPS database (Navtech), Google street view, data from community, organizations and federal databases, original research
	Natural spaces including green space	Residential surrounding greenness, distance to nearest green and blue spaces	Land cover/use maps (e.g., Urban Atlas in Europe)
	Traffic and transport	Traffic load, distance to roads, public transport network	Land use maps, official data from municipalities
	Food environment	Fast food restaurants, healthy food places	Open source data such as OpenStreetMap, Private GPS database (Navtech)
	Social deprivation	Area-wide poverty	Data from community organizations and federal database (US census), original research
Global	Global forces	Immigration, urbanization, industrialization (or industrial decline), climate change, human trafficking, war, pandemic disease	Studies from OECD, WHO, and other international governance agencies, transnational NGOs such as OXFAM and Greenpeace, census data showing historical patterns of residential mobility
	Government policies	Environmental legislation and banned substances, Patterns of economic investment, prevention strategies	Budgetary priorities (e.g., relative spending on defence or health); state or county level rankings of community or environmental health (e.g., Robert Wood Johnson Foundations' County Health Rankings, National Conference of Environmental Legislators)
Biological responses Internal		– Genetic damage (following radioactive exposures)	COnsortium of METabolomics Studies (COMETS, [9]), the Pregnancy And Childhood Epigenetics (PACE, [10]) consortium for epigenetics
		– Biological responses to toxic exposures from smaller molecules (metabolomics), to larger molecules (proteome) and genetic products (transcriptomics and epigenomics)	
		– Gut and skin microbiome (bacteria and virus)	

[a]DEMOCOPHES [11], HBM4EU (https://www.hbm4eu.eu/), the IPCHEM web-based portal for European biomonitoring data (https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html) or HELIX [12]

chemicals, with different protocol for each group of chemicals (phthalates, polychlorinated biphenyls, metals, etc.). Although this appears to be a laborious process, it should be recognized that these methods have shown great developments in recent years and now allow us to measure hundreds of already established and suspected chemical risk factors with greater accuracy and without the still uncertain annotation of molecules that is inherent to "top-down" approaches [13]. The bottom-up approach has the advantage that the choice of exposures to include can be based on a large amount of available data both from the experimental field and from observational studies in humans (National Health and Nutrition Examination Survey (NHANES), EU population-based research projects: DEMOCOPHES [11], HBM4EU (https://www.hbm4eu.eu/), the IPCHEM web-based portal for European biomonitoring data (https://ipchem.jrc.ec.europa.eu/RDSIdiscovery/ipchem/index.html) or HELIX [12]). Decades of prior research and evidence can, in this framework, feed into the choice and measurement of exposures. Highly accurate and validated methods can then give the best exposure estimate for each exposure. As such, the bottom-up approach can provide solid epidemiological evidence.

On the other hand, conceptually, the "top-down" measurement of exposome signals through the analysis of many molecules present in a biospecimen in one single analytical sweep is an attractive prospect in exposome research. In line with this, untargeted metabolomics could potentially be a "game-changing" tool. In principle, it provides the most comprehensive possible description of small molecular composition including biomarkers of exogenous exposure as well endogenous metabolites that together comprise a major component of the internal exposome. This field is currently in a stage of rapid development, promising to be able to measure and annotate hundreds to thousands of small molecules in each analytical run and shed light on the "dark exposome" or "unknown" chemical risk factors for disease, i.e., those that have not yet been identified as suspected risk factors and for which no high-accuracy measurement tools are available. However, analytical technology still faces several challenges [13]. One of the main challenges in measuring the chemical exposome is covering the large range of compound abundance in the human body. The concentration of endogenous metabolites, food biomarkers, and drugs present in the blood can span around eight orders of magnitude; however, when combined with environmental pollutants, the required range for detecting all exposome compounds present in the body grows to over 10 orders of magnitude from femtomoles to millimoles [14]. This exceeds the linear dynamic range of modern mass spectrometers by 10,000–100,000-fold. However, recent developments in separation science, by increasing the resolution of the existing separation methods (Ultra Performance Liquid Chromatography) and augmenting the complexity of data to detect more compounds (ion mobility spectrometry: IMS) are addressing this problem. Another strategy has been the use of (ultra)high-resolution mass spectrometry (HR-MS) which allows to increase radically the number of detected features and mass resolution with instruments such as Orbitraps and Fourier-Transform Ion-cyclotron Resonance (FT) [15]. Another option is to remove high abundant

analytes and concentrate low abundant exposome compounds such as used in targeted analyses or semi-targeted with the integration of standard reference compounds that allow for quantification.

Finally, the development of mass spectral libraries and prediction tools promises improved annotation of metabolic features. Often called the "dark matter", the majority of features measured by untargeted MS cannot be annotated to a compound. For exposome studies, the remaining unidentified peaks could represent a novel class of pesticides or an unknown metabolized xenobiotic. More than 40 million compounds are listed in PubChem and ChemSpider but only 1/100 may have available spectra. This is further complexified by the plethora of transformations a compound may undergo after chemical or enzymatic modifications in the body or during the measurement. Metabolome-wide databases are expanding endogenous compound libraries with environmental toxicants, food contaminants and supplements, drugs as well as their biotransformation products (METLIN, [16], MassBank of North America (MoNA) http://massbank.us/, and Human Metabolome Database, HMDB, www.hmdb.ca). There are also specialist metabolic databases dedicated to biomarkers of exposure to environmental risk factors that were developed (e.g., http://exposome-explorer.iarc.fr/). In addition, there are other tools relying on compound characteristics other than just mass such as fragmentation patterns (tandem MS,MS/MS), exact mass (HR-MS) or ion mobility (collision cross section, CSS), aided by advanced computation and machine learning for spectral prediction, for helping identify compounds uncovered in untargeted analyses [17].

Recent advances in sensor technologies and personal monitors allow more accurate and exposome-wide measurements, similar to the bottom-up approach described above for the chemical exposome, in the ambient environment. A recent study demonstrated possible to collect information on biotic (biological) and abiotic (chemical) compounds simultaneously from environmental airborne particules. Participants wore air-pump devices with two filters, which were analysed respectively by Next-generation Sequencing and LC-MS technologies [18]. They found 2796 unique formulae of the chemical exposome, including the insect repellent diethyltoluamide (DEET), the pesticide omethoate, and the carcinogen diethylene glycol (DEG), which were present in every air sample taken by the participants at multiple time points and locations. The extent of the airborne biological diversity observed in this study was enormous with over 2500 species identified, 5.11M SNPs in 108 pan-domain species across all samples. This number is comparable to the number of SNPs evaluated in the human gut microbiome (101 bacterial species; 3.98M SNPs at the individual level, 10.3M for all samples) [19].

Based on the same analytical advances described in the previous section on metabolomics in biological samples, the external exposome also benefits from untargeted LC-MS technologies and the chemical composition of air, water, and even surfaces can be measured in an exposome-wide manner, as exemplified recently in workplace surfaces [20].

18.2.2 The General External Exposome

The general external exposome includes factors found in the milieu in which we live, to which everyone living in the same area is potentially exposed. Exposure to general outdoor factors at the local, community level includes climate, built environment, surrounding natural spaces, traffic, food environment, and social deprivation. They can be estimated from geospatial models, monitoring stations, satellite data, and land use databases, and assigned to study participants according to their geocoded home and school addresses using GIS platforms (see for more details in Table 18.1). Children are particularly vulnerable to their urban environment, a source of physical, chemical, and behavioural exposures (e.g., pollution, lack of green space, noise, physical activity) all of which have been associated with a variety of health outcomes including asthma, mental health, obesity, and cognitive development [21–25]. Schools are often urban exposome "hotspots" located in areas of high pollution or noise, compounded by high levels of car use during the "school run"; within the UK, around half of children are driven to school [25–27]. The pregnancy urban exposome has been described recently in six different European countries, highlighting the interaction between the social and urban sublevels [28].

The exposures covered by the general external exposome have been extended from the original Wild domain definition [8] to include exposures measured at a global level such as global forces and policies. Researchers from the field of social sciences [29], public health and health disparities research [30] have termed the social exposome or public health exposome to ensure the inclusion of key determinants of health beyond the molecular determinants of health as measured by the omics. The social determinants of health determine the conditions in which individuals are born, grow, play, work, and age, driven by policy prioritization and resource allocation throughout communities, countries, and the world. Global forces and systems include economic policies, social norms, environmental policies, and political systems. National or pan-national databases such as the American Community Survey (ACS) or European Surveys on Income and Living Condition (EUSILC) provide valuable information on a wide range of socio-economical topics including commute/journey and internet access.

18.2.3 Biological Responses: Incorporating Omics into Exposome Research

The internal exposome defined as the internal endogenous processes including epigenetic, gene expression, inflammation, and metabolism has often been assessed through high-throughput molecular omics methodologies such as genome-wide DNA methylation, transcriptomics, proteomics, and metabolomics. More recently,

analytical platforms such as HR-MS metabolomics in blood specimens are extending the coverage of internal exposures of potential health significance, whether they are derived from exogenous sources (e.g., pollutants, diet, drugs) or endogenous sources (e.g., hormones, human, and microbial metabolites) [14]. Knowledge on the biological responses to the exposome is important to improve biological plausibility, to understand how different exposures may affect health through common biological pathways, and, ultimately, to predict environmental health related disease.

In general, omics signatures may reflect both physiological responses to external exposures and internal signatures of health outcomes. Omics data are heterogeneous in terms of their dimension, nature, complexity, and stability/volatility. The strength and complexity of correlation structure are also heterogeneous across different types of omics data, varying from distance-driven correlation in the genome to more complex patterns in other omics, especially in metabolomics. Omics data are also highly sensitive to matrix selection (e.g., blood, urine, etc.) and experimental conditions (time of sample collection, sample storage, and analytical batches), and can therefore be affected by measurement error. For this reason, extensive data quality control prior to the main analysis is undergone to remove potential technical noise, hindering biological effects.

18.3 Describing the Exposome: Variability, Determinants, and Patterns Across the Population

An individual's exposome is made up of a great number of exposures, many of which are correlated, and which vary over time and across geographical locations [12, 28, 31, 32]. Understanding the patterns, correlations, and variabilities within the exposome is important for designing exposure assessment strategies and prevention. Moreover, transparent knowledge of the correlation structure of an exposome dataset is required for interpretation of associations [33, 34].

18.3.1 Correlation Structure of the Exposome

One outstanding challenge of interpreting exposure-disease associations is due to the dense correlations among all exposures [35]. According to the third Bradford Hill criteria for the causal link between the environment and disease [36] "We must not [...] over-emphasize the importance of the characteristic". "One-to-one relationships are not frequent. Indeed, I believe that multi-causation is generally more likely than single causation though possibly if we knew all the answer we might get back to a single factor". The exposome framework allows revising the specificity of exposure-health associations. Indeed, the dense correlation pattern between exposures makes it hard to identify the directionality of the potential causal relation

between exposures and outcome. The data-driven approach assumes little to no collinearity between environmental predictors, but it is almost impossible to select any single uncorrelated exposures out from the dense exposome. One strategy for addressing these analytical issues is to characterize the correlations in diverse cohorts to provide reference levels, within and between family of exposures or "field" to gauge biological significance of associations [37]. The correlation structure of large set of exposures has been described using data from the Spanish INMA (INfancia y Medio Ambiente) birth cohort [34], the US NHANES (National Health and Nutrition Examination Survey [33]), and LIFE (Longitudinal Investigation of Fertility and the Environment) Study [38], for example. More recently, the HELIX project has described the correlation structure of the exposome using over 100 environmental exposures that were assessed in 1301 pregnant women and their children across six European birth cohorts [32]. This exercise is a first step towards identifying mixture of exposures occurring together as a result of common routes of exposures, e.g., arsenic, mercury and perfluorinated compounds related to fish intake, or arising from common participant behaviour. This was exemplified in a recent exposome-metabolome wide association study in pregnant women, in whom cotinine levels were strongly associated with urinary coffee metabolites [39]. In addition, another potential common source of variation can be due to the nature of the measurement or exposure characteristics, e.g., lipophilic persistent pollutants measured in blood are associated with blood lipids and fat mass and therefore highly intercorrelated. Temporal, behavioural, and geographical variations can also be interpreted through this type of exercise. For example, in the LIFE study it was suggested that individual and sex (see Fig. 18.1c), rather than shared environment within the same household, could be a major factor influencing the covariation of the exposome [38]. Understanding the correlations of exposures has important analytical and sampling implications for exposomics research.

18.3.2 Temporal Variability of the Exposome

An important feature of the exposome is its longitudinal and dynamic nature, in contrast to the static and fixed genome. It has been proposed that a more viable, efficient, and economical approach for characterizing the human exposome is to produce a snapshot of the status of these exposome domains at critical periods in which changes in exposure are present as a consequence of developmental changes or altered patterns of behaviour (e.g., food habits). These snapshots act as representative measures for the critical periods from which they are taken, reflecting the exposures and downstream consequences at the individual level. Key different stages of life represent different sensitive windows to exposures and different exposome characteristics, in particular relevant to early life are these periods: preconception, pregnancy, infancy, childhood, adolescence. It is also crucial to understand the exposome temporal variability at an individual level to determine how single exposure measurements at a given time may represent average exposure over time and avoid exposure misclassi-

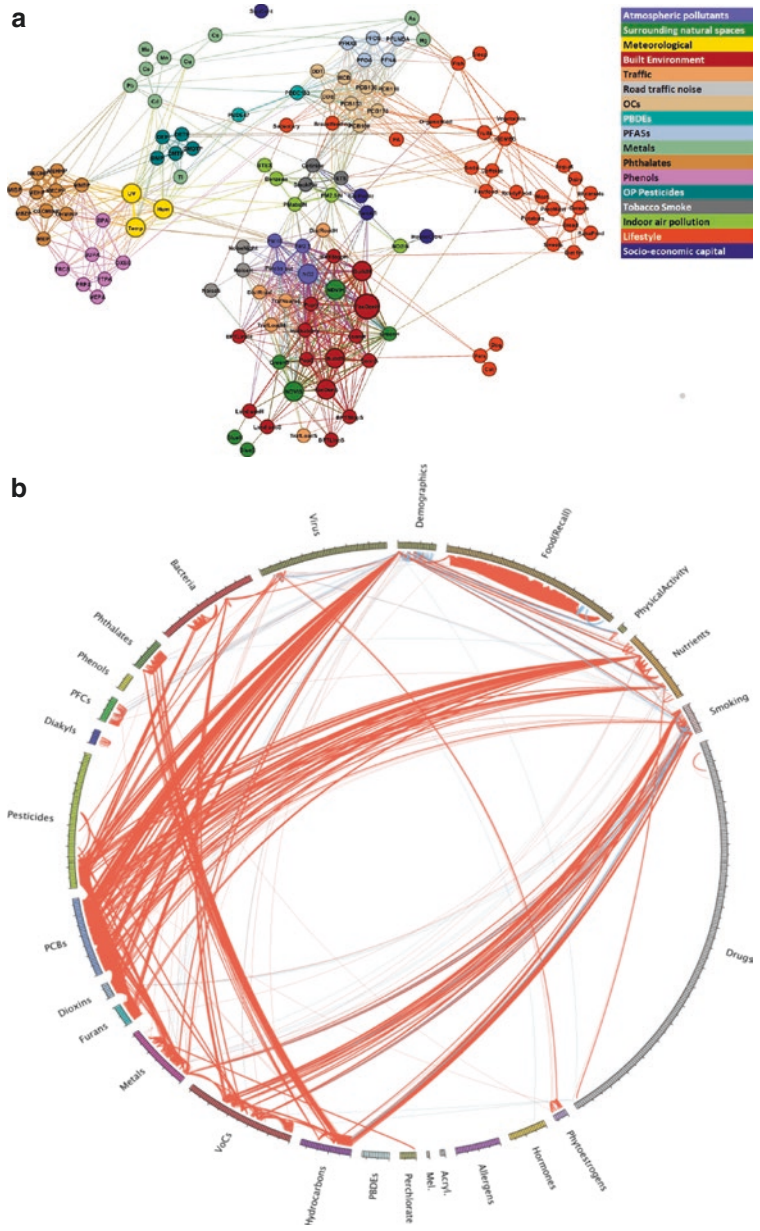


Fig. 18.1 Visualization of exposome correlation structures among different cohorts. (**a**) Network visualization of the exposome (122 exposure variables) during childhood in the HELIX study (7–11 years) from [32]; (**b**) Exposome (289 exposure variables) correlation globe in the NHANES dataset (reproduced from [40], https://pubs.acs.org/doi/10.1021/acs.est.8b01467, further permissions related to the material excerpted should be directed to the ACS); (**c**) Exposome (128 endocrine disrupting chemical) correlation globe showing the relationships of biomarkers between females, males, and couples (reproduced from [38])

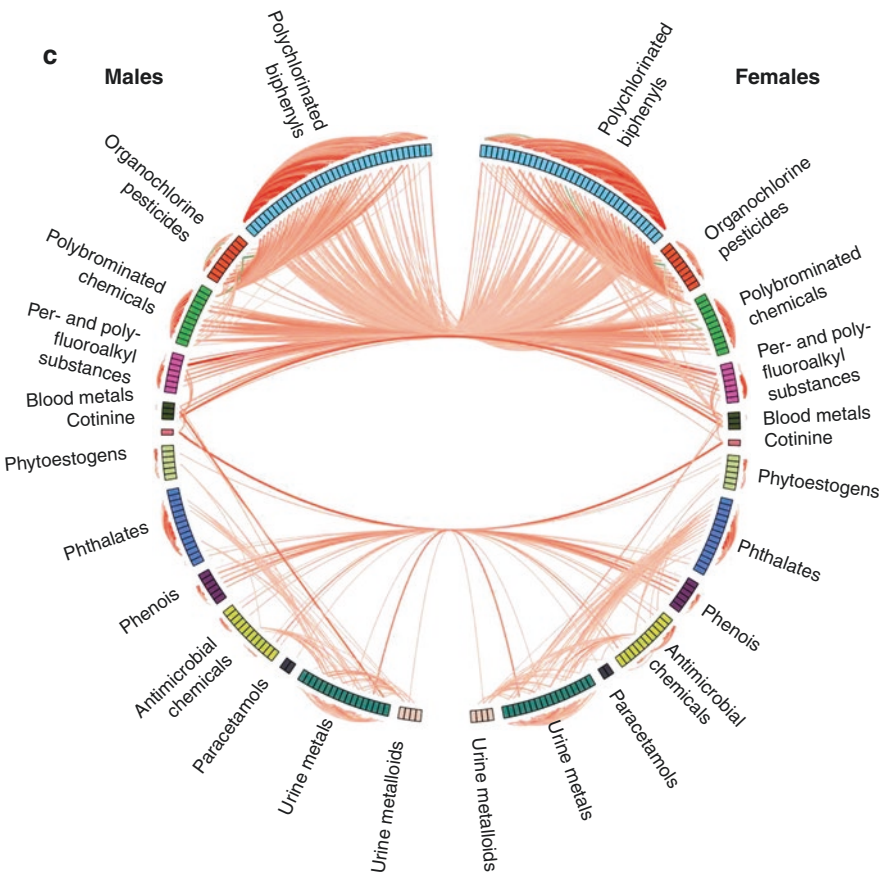


Fig. 18.1 (continued)

fication. This has been of particular concern for short-lived chemicals with high within-subject temporal variability such as phthalates, parabens, organophosphate pesticides, and phenols (e.g., bisphenols), with biological half-lives ranging from few hours to days. It has been estimated that studies using of a single biospecimen to assess the level of exposure biomarkers with high variability over time (intra-class coefficient of correlation of 0.2, corresponding approximately to what is observed with bisphenol A) might lead to an attenuation bias in the dose–response function by ~80% (the attenuation factor in the case of classical-type error corresponds to one minus the intra-class coefficient of correlation) [41]. In the case of a compound that is more stable over time, the attenuation bias will be lower (typically ~40% for specific phthalates metabolites). Bias can be limited by collecting several biospecimens per subject, and thereafter pooling them or using measurement error models. In a recent variability study in pregnant women and school children, it was calculated that a few dozen samples are required to accurately assess exposure over periods encompassing several trimesters or months for non-persistent pollutant [31].

Also for outdoor exposures, understanding individual mobility patterns and behaviour is crucial for accurate exposome assessment. New technologies and approaches that facilitate collection of personal real-time data (mobile app and personal sensors) offer strategies for capturing intra-individual variability. Human behaviour and use of time, referred to as time-activity patterns, for personal exposure assessment may indicate the distribution of time among activities and the factors that influence the degree of environmental contamination in the activities, and reflect the duration of contact during the activities [42]. Personal monitoring is particularly important since there is an inter- and intra-variability of individual's activities. The temporal resolution of the outdoor exposome can also provide information about the co-occurrence of certain exposures such as green spaces potential in reducing exposure to air pollution but increasing physical activity (in particular for less mobile groups such as pregnant women and children) [43, 44]. A recent study characterized the levels, variability, and correlations of personal environmental exposures, including air pollution, traffic-related noise, natural outdoor environments, and ultraviolet radiation, and levels of physical activity, of pregnant women and children in eight European cities, using a set of objective personal exposure assessment tools and Geographic information system (GIS) [44]. Similar to the chemical exposome assessment, it was shown that assessment of the personal exposures requires between 1 day of monitoring for surrounding greenness exposure to more than one year for UV-B exposure because of the high variability within participants.

18.4 Early-Life Exposome and Health

Until now, exposome-wide health studies with a bottom-up approach, using existing methods of exposure assessment with selected exposures of interest, have been conducted mainly on routinely collected or cross-sectional datasets, such as those examining environmental risk factors for type 2 diabetes [45], blood pressure [46], all causes mortality [47], pre-term birth [48], metabolic syndrome [49], and telomere length [50]. Most epidemiological research aiming at characterizing associations of early life environmental factors with health has naturally relied on pregnancy and birth cohorts [51]. The longitudinal cohort study design, starting during pregnancy and following children through adolescence and ultimately adulthood, is the most powerful observational study design to understand the role of early life environmental exposure and health throughout the life course. A few attempts to link fetal growth to more than one exposure family at a time have been made. Dadvand et al. [52] have related fetal growth to 10 "outdoor" environmental exposures (atmospheric pollutants such as particulate matter and nitrogen oxides, noise, heat, and road adjacent trees), while Lenters et al. [53, 54] considered exposure to a total of 16 biomarkers of phthalate metabolites, perfluoroalkyl acid, and organochlorine compounds in relation to fetal growth in 1250 newborns. More recently the HELIX project has shed light on the association between the urban prenatal exposome and child health [55] and prenatal and childhood exposomes and lung function [56].

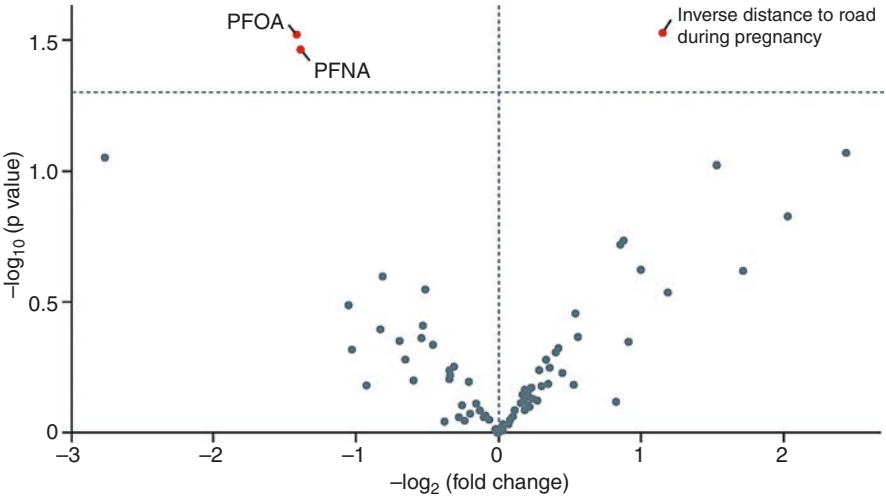


Fig. 18.2 Volcano plot of the association between the prenatal exposome and child lung function in the HELIX subcohort (N = 1033). The coefficient estimates for prenatal exposure variables versus p value (uncorrected for multiple hypothesis testing) in the ExWAS analysis of the exposure–FEV1% association. Coefficient estimates are given in FEV1% fold change for an IQR change in the given exposure, which was previously transformed to approach normality. The dashed horizontal line shows where $p = 0.05$. ExWAS = exposome-wide association study. FEV1% = forced expiratory volume in 1 s in per cent predicted. *PFNA* perfluorononanoate, *PFOA* perfluorooctanoate [56]

These studies are the first making all results from association tests (usually published in successive studies) explicit as displayed in Fig. 18.2, correcting for confounding by co-exposures and exposure misclassification.

From a methodological perspective, most previous studies relating the Exposome to health relied on the Environment-Wide Association Study (EWAS) [45], possibly followed by a multiple regression step. Several other regression-based methods exist and allow accounting for a potential joint action of multiple exposures on health. Sparse Partial Least Square (sPLS), for instance, has recently been used in a study of male fecundity [53], while Elastic Net (ENET) was used to link multiple environmental contaminants to birth weight [54]. The statistical performances of these various models in an Exposome context were systematically assessed [57]. Two- or three-way interactions between environmental exposures have been described in the literature and statistical methods to uncover interactions among a large set of exposures have been suggested. Exposome research in humans raises many challenges, including correcting for confounding by co-exposures and for differential exposure misclassification between factors with different within-subject variability, and improving statistical power or the identification of statistical or biological interactions between exposures. For example, no studies assessing persistent and non-persistent compounds attempted to correct for exposure misclassification, which is expected to be differential (i.e., of varying amplitude) between the least and most persistent compounds.

Omics can be considered as important exposures in their own right, fitting in the definition of the exposome as non-genetic causes of disease, or considered as external exposome mediators and thus both external and internal domains should be analysed side by side, with emphasis on interaction between and within domains. For instance, a study by Lenters assessed the impact of the gut microbiome diversity on childhood asthma together with persistent organic pollutant exposure and found that gut microbial diversity did not mediate the observed association between environmental chemicals and asthma [58]. In general, longitudinal cohort studies have been applying omics technologies in nested case–control studies to assess specific health outcomes or to study one particular external exposure effect such as tobacco smoking or arsenic but there are no example in the literature to our knowledge that present a more comprehensive exposome approach taking into account multiple external exposures and multiple omics signatures.

18.5 Future Perspectives

18.5.1 Exposome Measurements

Rapid technology development in analytical chemistry, new generation DNA sequencing, exposome databases, artificial intelligence, and machine learning are promising for exposure assessment in particular of unknown exposures. Untargeted, holistic analytical technologies are extending to environmental samples of air, water, and dust exposome capturing both the chemical and biological components [18]. However analytical technology still faces many challenges related to its ability to identify and accurately quantify many exogenous components of the exposome. Beyond analytical capabilities, the challenge remains for epidemiological studies to interpret the accuracy of single exposure measurements. Most chemicals of current regulatory concern comprise highly variable non-persistent chemical exposures. Measurements in single spot urine samples entail attenuation bias, which can amount to 80% in the case of compounds with very high within-subject variability such as bisphenol A [31, 41, 59]. Bias can be mitigated by within-subject pooling of many biospecimens, which avoids relying on spot samples. Similarly, improved accuracy of exposure estimates in the external and urban environment can be achieved by integrating information on how people move through their environment and on their personal exposome levels [44]. We have also learned that the exposome correlations are mainly found within exposure families and less so between families [32]; this means that one cannot capture the exposome by simply measuring a few exposure families (e.g., only air pollutants, noise, diet). We are thus in a situation where exposome measurement has to use complementary approaches to achieve both wide and accurate exposome coverage. Temporal, behavioural, and geographical variations should be covered by such measurements.

18.5.2 Data Analysis Methods

The specific characteristics of exposome data (high-dimensionality, complex correlations, temporal variability, hierarchical structure, repeated data points) require novel statistical and bioinformatics strategies. Until now statistical methods have considered multiple exposures separately (EWAS, [45]) or simultaneously (variable selection methods, [55]) or their joint effect (sPLS, [60]) but not their interactions. A comparison study of the existing methods to deal with interactions has reported that GLINTERNET (group LASSO) and DSA provide the best balance between sensitivity and specificity. However, to date, these methods only allow testing two-way and parametric interactions [61]. Methods to analyse combined effects of exposures related to health risk have also been proposed (e.g., Bayesian Kernel Regression, Bayesian Profile Regression) [62–64]. All strategies will need to take into account issues inherent to exposome data, such as collinearity between exposures, missing data, cohort effects, and exposure measurement errors that differ between exposures.

18.5.3 Large-Scale Exposome Research

In order to study numerous environmental pollutants and physical, lifestyle, and social risk factors and their combinations, as well as incorporating high-dimensional omics data, it is important for exposome research to look beyond one project and into the future, to have an efficient and large platform for generation of evidence and replication of findings. However, tools and data are at present scattered and information is still largely contained within scientific publications. Efforts have been made to harmonize and make available existing exposome data across multiple locations (both for omics and for ExWAS type studies, see, for example, HELIX database [51] or for metabolomics data, COMETS [9]). Multicentre exposome research should implement FAIR data infrastructure to enable findability, accessibility, interoperability, and (re)use of exposome data. The ongoing EU H2020 LifeCycle project, which brings together European pregnancy and child cohort studies into one harmonized data sharing platform, has started to implement these principles, building on $N = 80{,}000$ mother–child pairs at baseline in 15 cohorts from 10 countries spread across Northern, Eastern, Southern, and Western Europe. There have been other initiatives such as the Children's Health Exposure Analysis Resource (CHEAR) initiative in the USA, creating access to standardized laboratory tools for exposome research in children's health studies, eventually ensuring the comparability and replication of findings. This initiative has just been expanded in 2019 and should also include a Data Repository, Analysis and Science Center.

References

1. Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomark Prev. 2005;14:1847–50. https://doi.org/10.1158/1055-9965.EPI-05-0456.
2. Barton H, Grant M. A health map for the local human habitat. J R Soc Promot Heal. 2006;126:252–3.
3. Blane D, Kelly-Irving M, D'Errico A, Bartley M, Montgomery S. Social-biological transitions: how does the social become biological? Longitud Life Course Stud Int J. 2013;3:136–46. https://doi.org/10.14301/llcs.v4i2.236.
4. Kuh D, New Dynamics of Ageing (NDA) Preparatory Network. A life course approach to healthy aging, frailty, and capability. J Gerontol A Biol Sci Med Sci. 2007;62:717–21.
5. Blake-Lamb TL, Locks LM, Perkins ME, Woo Baidal JA, Cheng ER, Taveras EM. Interventions for childhood obesity in the first 1,000 days a systematic review. Am J Prev Med. 2016;50:780–9. https://doi.org/10.1016/j.amepre.2015.11.010.
6. Claus SP, Guillou H, Ellero-Simatos S. The gut microbiota: a major player in the toxicity of environmental pollutants? Biofilms Microbiomes. 2016;2:16003. https://doi.org/10.1038/npjbiofilms.2016.3.
7. Hsueh Y-M, Chen W-J, Lee C-Y, Chien S-N, Shiue H-S, Huang S-R, Lin M-I, Mu S-C, Hsieh R-L. Association of arsenic methylation capacity with developmental delays and health status in children: a prospective case–control trial. Sci Rep. 2016;6:37287. https://doi.org/10.1038/srep37287.
8. Wild CP. The exposome: from concept to utility. Int J Epidemiol. 2012;41:24–32. https://doi.org/10.1093/ije/dyr236.
9. Yu B, Zanetti KA, Temprosa M, Albanes D, Appel N, Barrera CB, Ben-Shlomo Y, Boerwinkle E, Casas JP, Clish C, Dale C, Dehghan A, Derkach A, Eliassen AH, Elliott P, Fahy E, Gieger C, Gunter MJ, Harada S, Harris T, Herr DR, Herrington D, Hirschhorn JN, Hoover E, Hsing AW, Johansson M, Kelly RS, Khoo CM, Kivimäki M, Kristal BS, Langenberg C, Lasky-Su J, Lawlor DA, Lotta LA, Mangino M, Le Marchand L, Mathé E, Matthews CE, Menni C, Mucci LA, Murphy R, Oresic M, Orwoll E, Ose J, Pereira AC, Playdon MC, Poston L, Price J, Qi Q, Rexrode K, Risch A, Sampson J, Seow WJ, Sesso HD, Shah SH, Shu X-O, Smith GCS, Sovio U, Stevens VL, Stolzenberg-Solomon R, Takebayashi T, Tillin T, Travis R, Tzoulaki I, Ulrich CM, Vasan RS, Verma M, Wang Y, Wareham NJ, Wong A, Younes N, Zhao H, Zheng W, Moore SC. The Consortium of Metabolomics Studies (COMETS): metabolomics in 47 prospective cohort studies. Am J Epidemiol. 2019;188:991–1012. https://doi.org/10.1093/aje/kwz028.
10. Felix JF, Joubert BR, Baccarelli AA, Sharp GC, Almqvist C, Annesi-Maesano I, Arshad H, Baïz N, Bakermans-Kranenburg MJ, Bakulski KM, Binder EB, Bouchard L, Breton CV, Brunekreef B, Brunst KJ, Burchard EG, Bustamante M, Chatzi L, Cheng Munthe-Kaas M, Corpeleijn E, Czamara D, Dabelea D, Davey Smith G, De Boever P, Duijts L, Dwyer T, Eng C, Eskenazi B, Everson TM, Falahi F, Fallin MD, Farchi S, Fernandez MF, Gao L, Gaunt TR, Ghantous A, Gillman MW, Gonseth S, Grote V, Gruzieva O, Håberg SE, Herceg Z, Hivert M-F, Holland N, Holloway JW, Hoyo C, Hu D, Huang R-C, Huen K, Järvelin M-R, Jima DD, Just AC, Karagas MR, Karlsson R, Karmaus W, Kechris KJ, Kere J, Kogevinas M, Koletzko B, Koppelman GH, Küpers LK, Ladd-Acosta C, Lahti J, Lambrechts N, Langie SA, Lie RT, Liu AH, Magnus MC, Magnus P, Maguire RL, Marsit CJ, McArdle W, Melén E, Melton P, Murphy SK, Nawrot TS, Nisticò L, Nohr EA, Nordlund B, Nystad W, Oh SS, Oken E, Page CM, Perron P, Pershagen G, Pizzi C, Plusquin M, Raikkonen K, Reese SE, Reischl E, Richiardi L, Ring S, Roy RP, Rzehak P, Schoeters G, Schwartz DA, Sebert S, Snieder H, Sørensen TI, Starling AP, Sunyer J, Taylor JA, Tiemeier H, Ullemar V, Vafeiadi M, Van Ijzendoorn MH, Vonk JM, Vriens A, Vrijheid M, Wang P, Wiemels JL, Wilcox AJ, Wright RJ, Xu C-J, Xu Z, Yang IV, Yousefi

P, Zhang H, Zhang W, Zhao S, Agha G, Relton CL, Jaddoe VW, London SJ. Cohort profile: Pregnancy And Childhood Epigenetics (PACE) Consortium. Int J Epidemiol. 2018;47:22–3. https://doi.org/10.1093/ije/dyx190.

11. Schindler BK, Esteban M, Koch HM, Castano A, Koslitz S, Cañas A, Casteleyn L, Kolossa-Gehring M, Schwedler G, Schoeters G, Den HE, Sepai O, Exley K, Bloemen L, Horvat M, Knudsen LE, Joas A, Joas R, Biot P, Aerts D, Lopez A, Huetos O, Katsonouri A, Maurer-Chronakis K, Kasparova L, Vrbík K, Rudnai P, Naray M, Guignard C, Fischer ME, Ligocka D, Janasik B, Reis MF, Namorado S, Pop C, Dumitrascu I, Halzlova K, Fabianova E, Mazej D, Tratnik JS, Berglund M, Jönsson B, Lehmann A, Crettaz P, Frederiksen H, Nielsen F, McGrath H, Nesbitt I, De Cremer K, Vanermen G, Koppen G, Wilhelm M, Becker K, Angerer J. The European COPHES/DEMOCOPHES project: towards transnational comparability and reliability of human biomonitoring results. Int J Hyg Environ Health. 2014;217:653–61. https://doi.org/10.1016/j.ijheh.2013.12.002.
12. Haug LS, Sakhi AK, Cequier E, Casas M, Maitre L, Basagana X, Andrusaityte S, Chalkiadaki G, Chatzi L, Coen M, de Bont J, Dedele A, Ferrand J, Grazuleviciene R, Gonzalez JR, Gutzkow KB, Keun H, McEachan R, Meltzer HM, Petraviciene I, Robinson O, Saulnier P-J, Slama R, Sunyer J, Urquiza J, Vafeiadi M, Wright J, Vrijheid M, Thomsen C. In-utero and childhood chemical exposome in six European mother-child cohorts. Environ Int. 2018;121:751–63. https://doi.org/10.1016/J.ENVINT.2018.09.056.
13. Dennis KK, Marder E, Balshaw DM, Cui Y, Lynes MA, Patti GJ, Rappaport SM, Shaughnessy DT, Vrijheid M, Barr DB. Biomonitoring in the era of the exposome. Environ Health Perspect. 2017;125:502–10. https://doi.org/10.1289/EHP474.
14. Rappaport SM, Barupal DK, Wishart D, Vineis P, Scalbert A. The blood exposome and its role in discovering causes of disease. Environ Health Perspect. 2014;122:769–74. https://doi.org/10.1289/ehp.1308015.
15. Jones DP. Sequencing the exposome: a call to action. Toxicol Rep. 2016;3:29–45. https://doi.org/10.1016/J.TOXREP.2015.11.009.
16. Warth B, Spangler S, Fang M, Johnson CH, Forsberg EM, Granados A, Martin RL, Domingo-Almenara X, Huan T, Rinehart D, Montenegro-Burke JR, Hilmers B, Aisporna A, Hoang LT, Uritboonthai W, Benton HP, Richardson SD, Williams AJ, Siuzdak G. Exposome-scale investigations guided by global metabolomics, pathway analysis, and cognitive computing. Anal Chem. 2017;89:11505–13. https://doi.org/10.1021/acs.analchem.7b02759.
17. Dührkop K, Shen H, Meusel M, Rousu J, Böcker S. Searching molecular structure databases with tandem mass spectra using CSI: FingerID. Proc Natl Acad Sci U S A. 2015;112:12580–5. https://doi.org/10.1073/pnas.1509788112.
18. Jiang C, Wang X, Li X, Inlora J, Wang T, Liu Q, Snyder M. Dynamic human environmental exposome revealed by longitudinal personal monitoring. Cell. 2018;175:277–91. https://doi.org/10.1016/j.cell.2018.08.060.
19. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, Waller A, Mende DR, Kultima JR, Martin J, Kota K, Sunyaev SR, Weinstock GM, Bork P. Genomic variation landscape of the human gut microbiome. Nature. 2012;493:45–50. https://doi.org/10.1038/nature11711.
20. McCall L-I, Anderson VM, Fogle RS, Haffner JJ, Hossain E, Liu R, Ly AH, Ma H, Nadeem M, Yao S. Characterization of the workplace chemical exposome using untargeted LC-MS/MS: a case study. bioRxiv: 541813. 2019. https://doi.org/10.1101/541813.
21. de Bont J, Casas M, Barrera-Gómez J, Cirach M, Rivas I, Valvi D, Álvarez M, Dadvand P, Sunyer J, Vrijheid M. Ambient air pollution and overweight and obesity in school-aged children in Barcelona, Spain. Environ Int. 2019;125:58–64. https://doi.org/10.1016/j.envint.2019.01.048.
22. Forns J, Dadvand P, Esnaola M, Alvarez-Pedrerol M, López-Vicente M, Garcia-Esteban R, Cirach M, Basagaña X, Guxens M, Sunyer J. Longitudinal association between air pollution exposure at school and cognitive development in school children over a period of 3.5 years. Environ Res. 2017;159:416–21. https://doi.org/10.1016/j.envres.2017.08.031.

23. Khreis H, Kelly C, Tate J, Parslow R, Lucas K, Nieuwenhuijsen M. Exposure to traffic-related air pollution and risk of development of childhood asthma: a systematic review and meta-analysis. Environ Int. 2017;100:1–31. https://doi.org/10.1016/j.envint.2016.11.012.
24. McEachan RRC, Yang TC, Roberts H, Pickett KE, Arseneau-Powell D, Gidlow CJ, Wright J, Nieuwenhuijsen M. Availability, use of, and satisfaction with green space, and children's mental wellbeing at age 4 years in a multicultural, deprived, urban area: results from the Born in Bradford Cohort Study. Lancet Planet Health. 2018;2:e244–54. https://doi.org/10.1016/S2542-5196(18)30119-0.
25. Stansfeld S, Clark C. Health effects of noise exposure in children. Curr Environ Health Rep. 2015;2:171–8. https://doi.org/10.1007/s40572-015-0044-1.
26. Kmietowicz Z. Air pollution: ban schools and other children's facilities from hotspots, say campaigners. BMJ. 2018;363:k4489. https://doi.org/10.1136/bmj.k4489.
27. UK Government. National Travel Survey Collection. Statistics and data about the National Travel Survey, based on a household survey to monitor trends in personal travel. 2018.
28. Robinson O, Tamayo I, de Castro M, Valentin A, Giorgis-Allemand L, Hjertager Krog N, Marit Aasvang G, Ambros A, Ballester F, Bird P, Chatzi L, Cirach M, Dėdelė A, Donaire-Gonzalez D, Gražuleviciene R, Iakovidis M, Ibarluzea J, Kampouri M, Lepeule J, Maitre L, McEachan R, Oftedal B, Siroux V, Slama R, Stephanou EG, Sunyer J, Urquiza J, Vegard Weyde K, Wright J, Vrijheid M, Nieuwenhuijsen M, Basagaña X. The urban exposome during pregnancy and its socioeconomic determinants. Environ Health Perspect. 2018;126:077005. https://doi.org/10.1289/EHP2862.
29. Senier L, Brown P, Shostak S, Hanna B. The socio-exposome: advancing exposure science and environmental justice in a post-genomic era. Environ Sociol. 2016;3(2):107–21.
30. Juarez PD. Sequencing the public health exposome: a proposed taxonomy for standardizing environmental exposures of the natural, built, social and policy environments. In: Macherone SDA, editor. Unraveling the exposome: a practical review. Cham: Springer; 2018. p. 23–61.
31. Casas M, Basagaña X, Sakhi AK, Haug LS, Philippat C, Granum B, Manzano-Salgado CB, Brochot C, Zeman F, de Bont J, Andrusaityte S, Chatzi L, Donaire-Gonzalez D, Giorgis-Allemand L, Gonzalez JR, Gracia-Lavedan E, Grazuleviciene R, Kampouri M, Lyon-Caen S, Pañella P, Petraviciene I, Robinson O, Urquiza J, Vafeiadi M, Vernet C, Waiblinger D, Wright J, Thomsen C, Slama R, Vrijheid M. Variability of urinary concentrations of non-persistent chemicals in pregnant women and school-aged children. Environ Int. 2018;121:561–73. https://doi.org/10.1016/j.envint.2018.09.046.
32. Tamayo-Uria I, Maitre L, Thomsen C, Nieuwenhuijsen MJ, Chatzi L, Siroux V, Aasvang GM, Agier L, Andrusaityte S, Casas M, de Castro M, Dedele A, Haug LS, Heude B, Grazuleviciene R, Gutzkow KB, Krog NH, Mason D, McEachan RRC, Meltzer HM, Petraviciene I, Robinson O, Roumeliotaki T, Sakhi AK, Urquiza J, Vafeiadi M, Waiblinger D, Warembourg C, Wright J, Slama R, Vrijheid M, Basagaña X. The early-life exposome: description and patterns in six European countries. Environ Int. 2019;123:189–200. https://doi.org/10.1016/j.envint.2018.11.067.
33. Patel CJ, Ioannidis JPA, Cullen MR, Rehkopf DH. Systematic assessment of the correlations of household income with infectious, biochemical, physiological, and environmental factors in the United States, 1999–2006. Am J Epidemiol. 2015;181:171–9. https://doi.org/10.1093/aje/kwu277.
34. Robinson O, Vrijheid M. The pregnancy exposome. Curr Environ Health Rep. 2015;2:204–13. https://doi.org/10.1007/s40572-015-0043-2.
35. Ioannidis JPA. Exposure-wide epidemiology: revisiting Bradford Hill. Stat Med. 2016;35:1749–62. https://doi.org/10.1002/sim.6825.
36. Hill AB. The environment and disease: association or causation? Proc R Soc Med. 1965;58:295–300.
37. Patel CJ, Ioannidis JPA. Placing epidemiological results in the context of multiplicity and typical correlations of exposures. J Epidemiol Community Health. 2014;68:1096–100. https://doi.org/10.1136/jech-2014-204195.

38. Chung MK, Kannan K, Louis GM, Patel CJ. Toward capturing the exposome: exposure biomarker variability and coexposure patterns in the shared environment. Environ Sci Technol. 2018;52:8801–10. https://doi.org/10.1021/acs.est.8b01467.
39. Maitre L, Robinson O, Martinez D, Toledano MB, Ibarluzea J, Marina LS, Sunyer J, Villanueva CM, Keun HC, Vrijheid M, Coen M. Urine metabolic signatures of multiple environmental pollutants in pregnant women: an exposome approach. Environ Sci Technol. 2018;52:13469–80. https://doi.org/10.1021/acs.est.8b02215.
40. Patel CJ, Manrai AK. Development of exposome correlation globes to map out environment-wide associations. Pac Symp Biocomput. 2015;20:231–42.
41. Perrier F, Giorgis-Allemand L, Slama R, Philippat C. Within-subject pooling of biological samples to reduce exposure misclassification in biomarker-based studies. Epidemiology. 2016;27:378–88. https://doi.org/10.1097/EDE.0000000000000460.
42. World Health Organization (WHO). Principles of characterizing and applying human exposure models. Geneva: WHO; 2013.
43. Dadvand P, de Nazelle A, Triguero-Mas M, Schembari A, Cirach M, Amoly E, Figueras F, Basagaña X, Ostro B, Nieuwenhuijsen M. Surrounding greenness and exposure to air pollution during pregnancy: an analysis of personal monitoring data. Environ Health Perspect. 2012;120:1286–90. https://doi.org/10.1289/ehp.1104609.
44. Donaire-Gonzalez D, Curto A, Valentín A, Andrusaityte S, Basagaña X, Casas M, Chatzi L, de Bont J, de Castro M, Dedele A, Granum B, Grazuleviciene R, Kampouri M, Lyon-Caen S, Manzano-Salgado CB, Aasvang GM, McEachan R, Meinhard-Kjellstad CH, Michalaki E, Pañella P, Petraviciene I, Schwarze PE, Slama R, Robinson O, Tamayo-Uria I, Vafeiadi M, Waiblinger D, Wright J, Vrijheid M, Nieuwenhuijsen MJ. Personal assessment of the external exposome during pregnancy and childhood in Europe. Environ Res. 2019;74:95–104. https://doi.org/10.1016/J.ENVRES.2019.04.015.
45. Patel CJ, Bhattacharya J, Butte AJ. An Environment-Wide Association Study (EWAS) on type 2 diabetes mellitus. PLoS One. 2010;5:e10746. https://doi.org/10.1371/journal.pone.0010746.
46. Tzoulaki I, Patel CJ, Okamura T, Chan Q, Brown IJ, Miura K, Ueshima H, Zhao L, Van Horn L, Daviglus ML, Stamler J, Butte AJ, Ioannidis JPA, Elliott P. A nutrient-wide association study on blood pressure. Circulation. 2012;126:2456–64. https://doi.org/10.1161/CIRCULATIONAHA.112.114058.
47. Patel CJ, Rehkopf DH, Leppert JT, Bortz WM, Cullen MR, Chertow GM, Ioannidis JP. Systematic evaluation of environmental and behavioural factors associated with all-cause mortality in the United States National Health and Nutrition Examination Survey. Int J Epidemiol. 2013;42:1795–810. https://doi.org/10.1093/ije/dyt208.
48. Patel CJ, Yang T, Hu Z, Wen Q, Sung J, El-Sayed YY, Cohen H, Gould J, Stevenson DK, Shaw GM, Ling XB, Butte AJ. Investigation of maternal environmental exposures in association with self-reported preterm birth. Reprod Toxicol. 2014;45:1–7. https://doi.org/10.1016/j.reprotox.2013.12.005.
49. Lind PM, Risérus U, Salihovic S, van BB, Lind L. An environmental wide association study (EWAS) approach to the metabolic syndrome. Environ Int. 2013;55:1–8. https://doi.org/10.1016/j.envint.2013.01.017.
50. Patel CJ, Manrai AK, Corona E, Kohane IS. Systematic correlation of environmental exposure and physiological and self-reported behaviour factors with leukocyte telomere length. Int J Epidemiol. 2016;46:dyw043. https://doi.org/10.1093/ije/dyw043.
51. Maitre L, de Bont J, Casas M, Robinson O, Aasvang GM, Agier L, Andrušaitytė S, Ballester F, Basagaña X, Borràs E, Brochot C, Bustamante M, Carracedo A, de Castro M, Dedele A, Donaire-Gonzalez D, Estivill X, Evandt J, Fossati S, Giorgis-Allemand L, Gonzalez RJ, Granum B, Grazuleviciene R, Bjerve Gützkow K, Småstuen Haug L, Hernandez-Ferrer C, Heude B, Ibarluzea J, Julvez J, Karachaliou M, Keun HC, Hjertager Krog N, Lau C-HE, Leventakou V, Lyon-Caen S, Manzano C, Mason D, McEachan R, Meltzer HM, Petraviciene

I, Quentin J, Roumeliotaki T, Sabido E, Saulnier P-J, Siskos AP, Siroux V, Sunyer J, Tamayo I, Urquiza J, Vafeiadi M, van Gent D, Vives-Usano M, Waiblinger D, Warembourg C, Chatzi L, Coen M, van den Hazel P, Nieuwenhuijsen MJ, Slama R, Thomsen C, Wright J, Vrijheid M. Human Early Life Exposome (HELIX) study: a European population-based exposome cohort. BMJ Open. 2018;8:e021311. https://doi.org/10.1136/bmjopen-2017-021311.

52. Dadvand P, Ostro B, Figueras F, Foraster M, Basagaña X, Valentín A, Martinez D, Beelen R, Cirach M, Hoek G, Jerrett M, Brunekreef B, Nieuwenhuijsen MJ. Residential proximity to major roads and term low birth weight. Epidemiology. 2014;25:518–25. https://doi.org/10.1097/EDE.0000000000000107.
53. Lenters V, Portengen L, Rignell-Hydbom A, Jönsson BAG, Lindh CH, Piersma AH, Toft G, Bonde JP, Heederik D, Rylander L, Vermeulen R. Prenatal phthalate, perfluoroalkyl acid, and organochlorine exposures and term birth weight in three birth cohorts: multi-pollutant models based on elastic net regression. Environ Health Perspect. 2015;124:365–72. https://doi.org/10.1289/ehp.1408933.
54. Lenters V, Portengen L, Smit LAM, Jönsson BAG, Giwercman A, Rylander L, Lindh CH, Spanò M, Pedersen HS, Ludwicki JK, Chumak L, Piersma AH, Toft G, Bonde JP, Heederik D, Vermeulen R. Phthalates, perfluoroalkyl acids, metals and organochlorines and reproductive function: a multipollutant assessment in Greenlandic, Polish and Ukrainian men. Occup Environ Med. 2015;72:385–93. https://doi.org/10.1136/oemed-2014-102264.
55. Nieuwenhuijsen MJ, Agier L, Basagaña X, Urquiza J, Tamayo-Uria I, Giorgis-Allemand L, Robinson O, Siroux V, Maitre L, de Castro M, Valentin A, Donaire-Gonzalez D, Dadvand P, Aasvang GM, Vrijheid M, Slama R. Influence of the urban exposome on birth weight. Environ Health Perspect. 2019;127(4):47007. https://doi.org/10.1289/EHP3971.
56. Agier L, Basagaña X, Maitre L, Granum B, Bird PK, Casas M, Oftedal B, Wright J, Andrusaityte S, de Castro M, Cequier E, Chatzi L, Donaire-Gonzalez D, Grazuleviciene R, Haug LS, Sakhi AK, Leventakou V, McEachan R, Nieuwenhuijsen M, Petraviciene I, Robinson O, Roumeliotaki T, Sunyer J, Tamayo-Uria I, Thomsen C, Urquiza J, Valentin A, Slama R, Vrijheid M, Siroux V. Early-life exposome and lung function in children in Europe: an analysis of data from the longitudinal, population-based HELIX cohort. Lancet Planet Health. 2019;3:e81–92. https://doi.org/10.1016/S2542-5196(19)30010-5.
57. Agier L, Portengen L, Chadeau-Hyam M, Basagaña X, Giorgis-Allemand L, Siroux V, Robinson O, Vlaanderen J, González JR, Nieuwenhuijsen MJ, Vineis P, Vrijheid M, Slama R, Vermeulen R. A systematic comparison of linear regression–based statistical methods to assess exposome-health associations. Environ Health Perspect. 2016;124(12):1848–56.
58. Lenters V. Assessing our multi-pollutant burden: environmental chemical exposures and reproductive and child health. Utrecht: Utrecht University; 2017.
59. Vernet C, Philippat C, Calafat AM, Ye X, Lyon-Caen S, Siroux V, Schisterman EF, Slama R. Within-day, between-day, and between-week variability of urinary concentrations of phenol biomarkers in pregnant women. Environ Health Perspect. 2018;126:037005. https://doi.org/10.1289/EHP1994.
60. Jain P, Vineis P, Liquet B, Vlaanderen J, Bodinier B, van Veldhoven K, Kogevinas M, Athersuch TJ, Font-Ribera L, Villanueva CM, Vermeulen R, Chadeau-Hyam M. A multivariate approach to investigate the combined biological effects of multiple exposures. J Epidemiol Community Health. 2018;72:564–71. https://doi.org/10.1136/jech-2017-210061.
61. Barrera-Gómez J, Agier L, Portengen L, Chadeau-Hyam M, Giorgis-Allemand L, Siroux V, Robinson O, Vlaanderen J, González JR, Nieuwenhuijsen M, Vineis P, Vrijheid M, Vermeulen R, Slama R, Basagaña X. A systematic comparison of statistical methods to detect interactions in exposome-health associations. Environ Health. 2017;16:74. https://doi.org/10.1186/s12940-017-0277-6.
62. Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, Godleski JJ, Coull BA. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics. 2015;16:493–508. https://doi.org/10.1093/biostatistics/kxu058.

63. Molitor J, Papathomas M, Jerrett M, Richardson S. Bayesian profile regression with an application to the National Survey of Children's Health. Biostatistics. 2010;11:484–98. https://doi.org/10.1093/biostatistics/kxq013.
64. Stafoggia M, Breitner S, Hampel R, Basagaña X. Statistical approaches to address multi-pollutant mixtures and multiple exposures: the state of the science. Curr Environ Health Rep. 2017;4:481–90. https://doi.org/10.1007/s40572-017-0162-z.

Chapter 19
Gene–Environment Interactions to Detect Adverse Health Effects on the Next Generation

Fumihiro Sata, Sumitaka Kobayashi, and Reiko Kishi

Abstract We reviewed epidemiological studies of gene–environment interactions to detect adverse health effects of environmental chemicals on the next generation in 2008, more than 10 years ago. Since then, researches on gene–environment interactions have continued via small-scale epidemiological studies seeking to elucidate associations between tobacco and environmental chemicals and candidate genes such as those encoding metabolic enzymes. In the last 10 years, extensive innovation in research designs and methods, accompanied by recent rapid advances in analytical technologies, has occurred. Specifically, genome-wide association studies (GWASs) and epigenome-wide association studies (EWASs) have become mainstream in genome cohort studies using advanced genomics and epigenomics. These have made it possible to better understand the genetic basis of diseases. Furthermore, in addition to GWASs and meta-analyses, Mendelian randomization has emerged as a GWAS-based theoretical method for environmental risk assessment that uses genetic factors associated with environmental factors. Although the concept of exposome was initially proposed as an improved tool to quantify total environmental contributions, linking it with genomics is expected to additionally enable the elucidation of the origins of multiple complex diseases. We have reviewed researches on gene–environment interactions and discussed recently developed approaches, such as GWAS, Mendelian randomization, and exposome linked with genomics, for evaluating genetic susceptibility.

Keywords Susceptibility · Genome-wide association study (GWAS) · Meta-analysis · Non-communicable disease (NCD) · Developmental origins of health and disease (DOHaD)

F. Sata (✉)
Health Center, Chuo University, Tokyo, Japan

Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan
e-mail: fsata.16h@g.chuo-u.ac.jp

S. Kobayashi · R. Kishi
Center for Environmental and Health Sciences, Hokkaido University, Sapporo, Japan

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_19

Abbreviations

ADA1	Adenosine deaminase 1
AHR	Aromatic hydrocarbon receptor
CBS	Cystathionine beta-synthase
CHEAR	Child Health Environmental Analysis Resource
CYP	Cytochrome P450
DOHaD	Developmental origins of health and disease
EGG	Early growth genetics
GST	Glutathione *S*-transferase
GSTTP1	GST theta pseudogene 1
EPHX1	Epoxide hydrolase 1
EWAS	Epigenome-wide association study
GWAS	Genome-wide association study
LHCGR	Luteinizing hormone/chorionic gonadotropin receptor
INHA	Inhibinα
IUGR	Intrauterine growth restriction
LBW	Low birth weight
MTHFD1	Methylenetetrahydrofolate dehydrogenase 1
MTHFR	5,10-Methylenetetrahydrofolate reductase
MTR	5-Methyltetrahydrofolate-homocysteine methyltransferase
MTRR	5-Methyltetrahydrofolate-homocysteine methyltransferase reductase
NAT2	*N*-acetyltransferase 2
NCD	Non-communicable disease
NQO1	NAD(P)H dehydrogenase
OGG1	8-Oxoguanine glycosylase
PB	Preterm birth
SGA	Small-for-gestational-age
SHMT1	Serine hydroxymethyltransferase 1
SNP	Single-nucleotide polymorphism
TGFBR1	Transforming growth factor-β receptor type 1
XRCC1	X-ray repair cross-complementing gene 1
XRCC3	X-ray repair cross-complementing gene 3

19.1 Introduction

In 2008, more than 10 years ago, we reviewed epidemiological studies of gene–environment interactions to detect the adverse health effects of environmental chemicals on the next generation [1]. As is well documented, numerous chemical compounds such as polycyclic aromatic hydrocarbons (PHA) in tobacco smoke are activated and detoxified by xenobiotic-metabolizing enzymes [1]. Xenobiotic-metabolizing genes appear to be influenced functionally by maternal smoking during pregnancy, which may be a significant risk factor for low birth weight (LBW)

and/or intrauterine growth restriction (IUGR) [1–4]. The Hokkaido Study, a pioneering work in this field, examined the effects of environmental factors together with a genetic predisposition on the health and development of about 20,000 children across the Hokkaido prefecture, the northern part of Japan, from the prenatal period onward [5, 6].

The last decade has witnessed major innovations in research design and methods, accompanied by recent advances in technologies for statistical and biological analyses and measurement [7–10]. In particular, genome-wide association studies (GWASs), including epigenome-wide association studies (EWASs), have become mainstream in genome cohort studies using advanced genomics and epigenomics techniques, which has made it possible to better understand the genetic basis of diseases [7]. Furthermore, Mendelian randomization is a fast-growing area that involves the analysis of genetic variants to assess the causal relationships between exposure and outcome [8]. The field of exposure science, termed exposome has emerged, which involves the study of mechanisms by which "non-genetic" exogenous and endogenous exposure influence the risk of disease [9, 10]; linking this field with genomics is expected to enable elucidation of the origins of multiple complex diseases [9]. The methods for evaluating gene–environment interactions have advanced rapidly because of the accumulation of large-scale data, termed big data. Using GWASs, we have obtained data for hundreds of complex traits across a wide range of domains, including common diseases and quantitative traits that are risk factors for diseases, enabling us to better define the relative role of genes and the environment in disease risk [7]. Examination of the impact of early life exposure and maternal physical and mental conditions during pregnancy is a topic of interest for investigation by exposomics, using environmental factors and biological samples from cohorts [9, 10]. Thus, it is recognized that numerous human diseases arise from the complex interplay between environmental exposure and host susceptibilities.

In the present work, we introduce recent progress in evaluating gene–environment interactions in large-scale genome epidemiological studies as well as in small-scale single studies targeting candidate genes.

19.2 Infant Birth Size, Including Low Birth Weight (LBW), Preterm Birth (PB), Small-for-Gestational-Age (SGA), Gestational Age, and Intrauterine Growth Restriction (IUGR) in Relation to Maternal Smoking

It is known that maternal smoking during pregnancy may lead to a reduction in infant birth size including LBW, PB, SGA, gestational age, and IUGR. In recent years, it has been found that this association is modified by genetic factors. In research studies published up to 2018, this association is reported to be modified by maternal genotypes of genes encoding the xenobiotic receptor (aromatic hydrocarbon receptor [*AHR*]), enzymes (cytochrome P450 [*CYP*] *1A1*, *CYP2A6*, *CYP2E1*, glutathione *S*-transferase [*GST*] mu 1 [*GSTM1*], GST theta 1 [*GSTT1*], GST theta 2 [*GSTT2*],

epoxide hydrolase 1 [*EPHX1*], 5,10-methylenetetrahydrofolate reductase [*MTHFR*], and NAD(P)H dehydrogenase [*NQO1*]), DNA repair proteins (X-ray repair cross-complementing gene 1 [*XRCC1*], *XRCC3*, 8-oxoguanine glycosylase [*OGG1*]), oncogenes (*MDM4*), and tumor suppressor genes (*TP53*) [11–33] (Table 19.1). Moreover, this association has been observed to be modified by fetal genotypes of genes encoding xenobiotic-metabolizing enzymes (*GSTT1* and *NQO1*) and cell-division-related genes (adenosine deaminase 1 [*ADA1*]) [14, 16, 29]. Most of these studies assessed the smoking status of pregnant women using a questionnaire. A few studies still evaluate the smoking status of pregnant women using objective indicators.

Although studies that have evaluated pregnant women by smoking status using objective indicators are limited, three previous reports have examined the effects of gene–environment interactions of maternal smoking on infant birth size using biomarkers [21, 24, 27]. Only one publication has examined the dose-dependent association of gene–environment interactions and maternal smoking with infant birth size among 3263 Japanese pregnant women enrolled in the prospective birth cohort of the Hokkaido Study on Environment and Children's Health. Without consideration of genotype, maternal passive smoker levels (plasma cotinine levels: 0.22–11.48 ng/mL) were associated with a mean reduction of 55–57 g in birth weight, and maternal active smoker levels (plasma cotinine levels: ≥11.49 ng/mL) were associated with a mean reduction of 93–171 g in birth weight, compared with that elicited by non-passive smoker levels (plasma cotinine levels: ≤0.21 ng/mL). When maternal *AHR* (rs2066853) genotypes were considered, active smoker levels were associated with a mean reduction in birth weight of up to 102 g compared with that caused by non-passive smoker levels among maternal AA genotypes. However, active smoker levels were associated with a mean reduction of 182–217 g compared with that associated with non-passive smoker levels among maternal GG genotypes. Differences have also been observed in maternal *XRCC1* (rs1799782) genotypes when examining the dose-dependent association between plasma cotinine levels and birth weight reduction (Fig. 19.1) [24]. Further, it has been shown that this association is not modified by maternal or fetal genotypes of genes encoding xenobiotic enzymes (*CYP1A2*, *CYP1B1*, cystathionine beta-synthase [*CBS*], GST theta pseudogene 1 [*GSTTP1*], methylenetetrahydrofolate dehydrogenase 1 [*MTHFD1*], 5-methyltetrahydrofolate-homocysteine methyltransferase [*MTR*], 5-methyltetrahydrofolate-homocysteine methyltransferase reductase [*MTRR*], *N*-acetyltransferase 2 [*NAT2*], and serine hydroxymethyltransferase 1 [*SHMT1*]), hormone-related factors and receptors (inhibinα [*INHA*], luteinizing hormone/chorionic gonadotropin receptor [*LHCGR*], and transforming growth factor-β receptor type 1 [*TGFBR1*]), or by none of these factors [21, 24, 29, 30, 32].

The following points of the problems should be recognized when evaluating gene–environment interactions of an association between maternal smoking and infant birth size. Some of these problems include: (1) differences in time of evaluation (e.g., first trimester of pregnancy or the 8th month of pregnancy), (2) differences in the questions used for assessment (e.g., cigarettes/day or a choice between yes and no), (3) differences in evaluation methods (e.g., the use of objective biomarkers or subjective questionnaires), (4) differences in study design (e.g., case–control study or prospective birth cohort study), and (5) differences in the location

Table 19.1 Smoking and birth outcomes: gene–environment interactions (only relevant)

Birth outcome	Environmental exposure	Maternal or fetal genetic polymorphism	Maternal or fetal risk genotype	Birth weight reduction (g)	Reference
Birth weight reduction	Smoking	*CYP1A1 MspI* (mother)	Aa or aa	−520	Wang et al. [11]
	Smoking	*GSTT1* (mother)	absent	−642	Wang et al. [11]
	Smoking	*AHR Arg554Lys* (mother)	Arg/Arg	−211	Sasaki et al. [12]
	Smoking	*CYP1A1 MspI* (mother)	m1/m2 or m2/m2	−170	Sasaki et al. [12]
	Smoking	*GSTM1* (mother)	Null	−171	Sasaki et al. [12]
	Smoking	*AHR Arg554Lys* (mother) and *CYP1A1 MspI* (mother)	Both Arg/Arg and m1/m2 or m2/m2	−315	Sasaki et al. [12]
	Smoking	*AHR Arg554Lys* (mother) and GSTM1 (mother)	Both m1/m2 or m2/m2 and null	−237	Sasaki et al. [12]
	Smoking	*NQO1 Pro185Ser* (mother)	Pro/pro	−199	Sasaki et al. [13]
	Smoking	*CYP2E1∗5* (mother)	c1/c1 or c1/c2	−195	Sasaki et al. [13]
	Smoking	*ADA* (infant)	ADA∗1	−255	Gloria-Bottini et al. [14]
	Smoking	*GSTM1* (mother) and *GSTT1* (mother)	Both null and null	−311.2	Grazuleviciene et al. [15]
	Smoking	*GSTT1* (mother)	Null	−190	Aagaard-Tillery et al. [16]
	Smoking	*GSTT1* (infant)	Null	−262	Aagaard-Tillery et al. [16]
	Smoking	*OGG1 Ser326Cys* (mother)	CG or GG	−406	Karahalil et al. [17]
	Smoking	*GSTM1* (mother) and *GSTT1* (mother)	Both null and null	−311.2	Danileviciute et al. [18]
	Smoking	*GSTT2* (mother)	Deletion	−284	Zheng et al. [19]
	Smoking	*MTHFR A1298C* (mother)	AA	−106.59	Yila et al. [20]
	Smoking (based on cotinine)	*AHR Arg554Lys* (mother)	GG	−56	Kobayashi et al. [21]

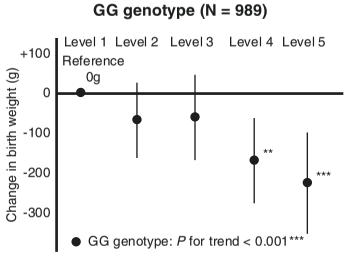
(continued)

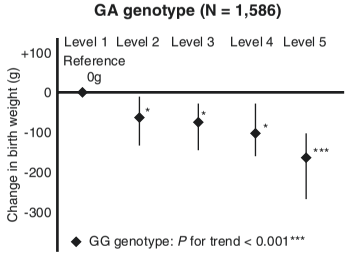
Table 19.1 (continued)

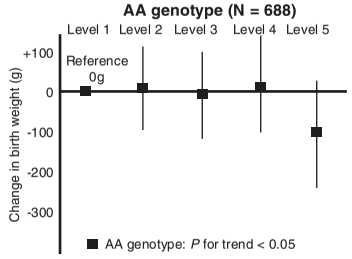
Birth outcome	Environmental exposure	Maternal or fetal genetic polymorphism	Maternal or fetal risk genotype	Birth weight reduction (g)	Reference
	Smoking (based on cotinine)	*CYP1A1 Ile462Val* (mother)	AG or GG	−62	Kobayashi et al. [21]
	Smoking (based on cotinine)	*XRCC1 Arg194Trp* (mother)	CT or TT	−59	Kobayashi et al. [21]
	Smoking (based on cotinine)	*XRCC1 Gln399Trp* (mother)	GA or AA	−46	Kobayashi et al. [21]
	Smoking (based on cotinine)	*AHR* (mother), *CYP1A1* (mother), and *XRCC1 Arg194Trp* (mother)	*AHR*-GG, *CYP1A1*-AG or GG, and *XRCC1*-CT or TT	−145	Kobayashi et al. [21]
	Environmental tobacco smoking (ETS)	*GSTT1* (mother)	Null	−236	Hong et al. [22]
	Passive smoking	*EPHX1 Tyr113His* (mother)	His/His	−315.6	Wu et al. [23]
	Cotinine (dose-dependent association)	*AHR Arg554Lys* (mother)	GG	Association observed	Kobayashi et al. [24]
	Cotinine (dose-dependent association)	*CYP1A1 Ile462Val* (mother)	GG	Association observed	Kobayashi et al. [24]
	Cotinine (dose-dependent association)	*XRCC1 Arg194Trp* (mother)	TT	Association observed	Kobayashi et al. [24]
Increased risk of SGA	Smoking	*CYP1A1* (mother)	*CYP1A1*∗*2A*	SGA risk Interaction observed	Infante-Rivard et al. [25]
	Smoking	*XRCC3 Thr241Met* (mother)	Thr/Met or Met/Met	Interaction observed	Infante-Rivard et al. [25]
	Smoking	*GSTT1* (infant)	Null	Interaction observed	Infante-Rivard et al. [25]
	Second-hand smoke (SHS)	*CYP2A6*∗*4* (mother)	∗1/∗4 or ∗4/∗4	Odds ratio = 1.98	Xie et al. [26]

	SHS	*CYP2A6*4* (mother) and *GSTT1* (mother)	Both *1/*4 or *4/*4 and absent	Odds ratio = 2.45	Xie et al. [26]
	Cotinine	*GSTM1* (mother)	Null	Odds ratio = 5.68	Huang et al. [27]
	Cotinine	*GSTT1* (mother)	Null	Odds ratio = 7.59	Huang et al. [27]
	Cotinine	*GSTM1* (mother) and *GSTT1* (mother)	Both null and null	Odds ratio = 8.90	Huang et al. [27]
Increased risk of IUGR	Smoking	*CYP1A1 MspI* (mother)	Aa/aa	IUGR risk	Delpisheh et al. [28]
				Odds ratio = 3.2	
	Smoking	*GSTM1* (mother)	Absent	Odds ratio = 4.1	Delpisheh et al. [28]
	Smoking	*GSTT1* (mother)	Absent	Odds ratio = 4.3	Delpisheh et al. [28]
	Smoking	*NQO1 Pro187Ser* (infant)		Association observed	Price et al. [29]
Increased risk of PB	Smoking	*GSTT1* (mother or infant)	Maternal null or infant null	PB risk	Nukui et al. [30]
				Odds ratio = 4.02	
	Smoking	*GSTT1* (mother and infant)	Both maternal null and infant null	Odds ratio = 7.15	Nukui et al. [30]
	Smoking	*CYP1A1 MspI* (mother) and *GSTT1* (mother)	Both Aa or aa and absent	Odds ratio = 5.8	Tsai et al. [31]
Increased risk of both IUGR and PB	Smoking	*MDM4* rs1090595 (mother)	AC	Both IUGR and PB risk	Huang et al. [32]
				Odds ratio = 1.38	
	Smoking	*TP53* (mother)	CT	Odds ratio = 1.66	Huang et al. [32]

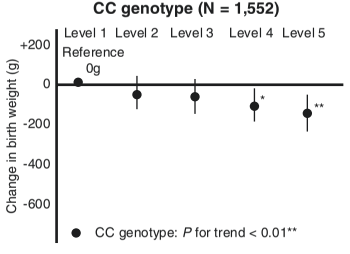
(i) *AHR* (G>A, Arg554Lys)

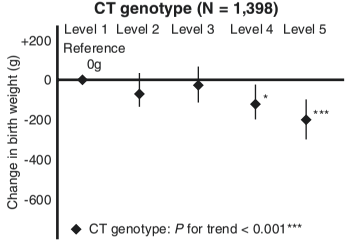


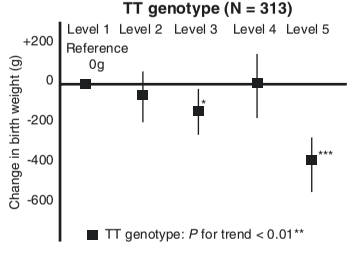




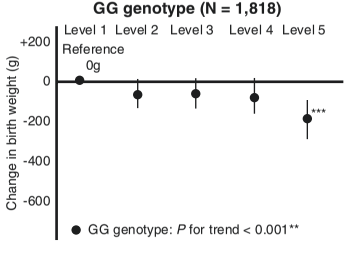
(ii) *XRCCI* (C>T, Arg194Trp)

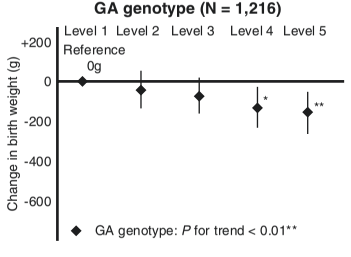


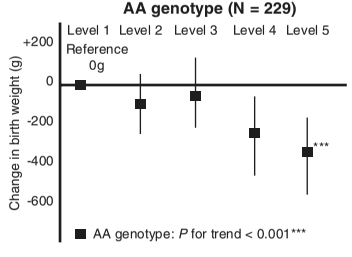




(iii) *XRCCI* (G>A, Arg399Gln)







of gene polymorphisms (e.g., rs4646903 or rs1048943 polymorphism of the *CYP1A1* gene). Furthermore, maternal exposure levels such as through active smoking or passive smoking (second-hand smoke; SHS) must be considered.

Finally, it is necessary to focus on the components of tobacco smoke, which contains about 4000 substances. Receptors bind the chemical substances contained in tobacco smoke and initiate intracellular reactions. Based on findings from animal studies and cell experiments, it is important to examine gene–environment interactions focusing on single-nucleotide polymorphisms (SNPs) of genes involved in biological mechanisms, e.g., tobacco smoke induces toxicity. Therefore, studies should aim to elucidate the molecular epidemiology underlying the association between maternal smoking and birth outcome to identify the receptors in either the mother or infant (or both) that modify the association between components of tobacco smoke and adverse health effects on infants and children.

19.3 Effects of Exposure to Other Chemicals on Infant Birth Size

In studies performed up to 2018, many substances that pregnant women are exposed to have been found to modulate gene–environment interactions in fetal growth. These include environmental pollutants (benzo(a)pyrene), disinfection by-products of drinking water (trihalomethanes, chloroforms, and haloacetic acids), fatty acids (cholesterols, triglyceride, and docosahexaenoic acid [DHA]), vitamins (carotenes, vitamin C, vitamin D, and vitamin E), beverage-derived substances (alcohol and caffeine), types of particle matters (PMs) (PMs of aerodynamic diameter <10 μm [PM_{10}], <2.5 μm [$PM_{2.5}$], and nitrogen oxides [NO_x]), metals (lead, mercury, and iron), short half-life chemicals (alkyl phosphates and phthalates), pesticides including those used in floriculture (organochlorines), and persistent organic pollutants (POPs; perfluoroalkyl substances (PFASs), and dioxins) [34–56] (Table 19.2). Reduced birth weight is caused by the association between PM_{10} levels and maternal *CYP1A1* genotype [34], caffeine levels and maternal *CYP1A2* genotype [37], benzo(a)pyrene levels and maternal *GSTP1* genotype [36], vitamin D levels and maternal genotypes of the gene encoding group-specific component (vitamin D binding protein) locus (GC) [38], cholesterol levels and maternal apolipoprotein E

Fig. 19.1 Association of maternal *AHR* (G>A, *Arg554Lys*; db SNP ID: rs2066853) and *XRCC1* (C>T, *Arg194Trp*; db SNP ID: rs1799782 and G>A, *Arg399Gln*; db SNP ID: rs25487) genotype with maternal cotinine levels in relation to infant birth weight ($n = 3263$) [24]. Ninety-five percent confidence intervals (CI). Maternal plasma cotinine levels: level 1, 0.12–0.21 ng/mL; level 2, 0.22–0.55 ng/mL; level 3, 0.56–11.48 ng/mL; level 4, 11.49–101.66 ng/mL; level 5, 101.67–635.25 ng/mL. Multiple linear regression models are adjusted for maternal age, height, weight before pregnancy, parity, alcohol intake during the first trimester of pregnancy, education level, annual household income, infant gender, and gestational age. β represents the change in infant birth weight (g) in comparison with level 1 as the reference. Dot represents β values (95% CI); $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Table 19.2 Other chemicals and birth outcomes: gene–environment interactions (only relevant)

Birth outcome	Environmental exposure	Maternal or fetal genetic polymorphism	Maternal or fetal risk genotype	Birth weight reduction (g)	Reference
Birth weight reduction	PM_{10}	*CYP1A1 NcoI* (mother)	Ile/Val or Val/Val	−489	Suh et al. [34]
	$PM_{2.5}$	*GSTP1* rs1695 (infant)	*GSTP1∗1B/1B*	−168	Slama et al. [35]
	$PM_{2.5}$	*CYP2D6* rs1800716 (infant)	*CYP2D6∗4/∗4*	−311	Slama et al. [35]
	Benzo(a)pyrene	*GSTP1 Ile105Val* (mother)	Ile/Val or Val/Val	−145.06	Duarte-Salles et al. [36]
	Benzo(a)pyrene	*GSTP1 Ile105Val* (infant)	Ile/Val or Val/Val	−130.79	Duarte-Salles et al. [36]
	Caffeine	*CYP1A2* C164A (mother)	AA	−277	Sasaki et al. [37]
	Vitamin D	GC rs12512631 (mother)	CC	−111.1	Chun et al. [38]
	Total cholesterol (TC)	*APOE* rs7412; rs429358 (mother)	*APOE* ε3ε4	Association observed	Ruiz et al. [39]
	Docosahexaenoic acid (DHA)	*FASD* rs174556 (mother)	CT or TT	Interaction observed	Molto-Puigmatti et al. [40]
	Lead	*HFE* rs1799635 (mother)	D	Interaction observed	Cantonwine et al.[41]
	Mercury	*GSTM1* (mother) and *GSTT1* (mother)	Both *GSTM1* null and *GSTT1* null	−102.8	Lee et al. [42]
	Iron	*GSTM1* (mother)	*GSTM1* null	Interaction observed	Hur et al. [43]
	HCH (organochlorine pesticide)	*GSTM1* (mother)	*GSTM1* null	Interaction observed	Sharma et al. [44]
	Endosulfan (organochlorine pesticide)	*CYP17A1* (mother)	A1/A2 or A2/A2	Interaction observed	Chand et al. [45]
	Perfluorinated compounds	*GSTM1* (mother)	Null	Interaction observed	Kwon et al. [46]
	Dioxins	*GSTM1* (mother)	Null	−346	Kobayashi et al. [47]
Gestational age reduction	NO_x	*IL-17A* G197A (mother)	GG	Gestational age reduction	Nansook et al. [48]
				−0.364	
	Dialkyl phosphate (DAP) metabolites	*PON1* Q192R (infant)	RR	Interaction observed	Harley et al. [49]

Increased risk of SGA	Alcohol	*ADH2* (infant)	*ADH2*∗*2* negative	SGA risk Odds ratio = 3.15	Arfsten et al. [50]
	Total trihalomethane (TTHM)	*CYP2E1* rs743535 (mother)	CC	Odds ratio = 1.1	Kogevinas et al. [51]
	Total trihalomethane (TTHM)	*GSTT1* (mother)	Null copy number variant	Odds ratio = 1.4	Kogevinas et al. [51]
	Trichloroacetic acid	*CYP17A1* rs491987 (infant)	1 or 2 variant allele	Odds ratio = 2.4	
Increased risk of LBW	Floriculture work	*PON1* Q192R (mother)	RR	LBW risk Interaction observed	Moreno-Banda et al. [52]
	Mono-2-ethylhexyl phthalate (MEHP)	*PON2* A148G (infant)	AG or GG	Odds ratio = 5.0	Xie et al. [53]
	Mono-*n*-butyl phthalate (MBP)	*PON2* A148G (infant)	AG or GG	Odds ratio = 2.6	Xie et al. [53]
	Total trihalomethane (TTHM)	*GSTM1* (mother)	Null	Interaction observed	Danileviciute et al. [54]
	Chloroform (CH)	*GSTM1* (mother)	Null	Interaction observed	Danileviciute et al. [54]
Increased risk of IUGR	Alcohol	*CYP17A1* (mother)	A1/A1	IUGR risk Odds ratio = 2.6	Delpisheh et al. [55]
	Total trihalomethane (TTHM)	*CYP2E1∗5* G1259C (infant)	1 or 2 variant alleles	Odds ratio = 13.20	Infante-Rivard et al. [56]

(*APOE*) and apolipoprotein C3 (*APOC3*) genotype [39], DHA levels and maternal fatty acid desaturase (*FADS*) genotype [40], lead levels and maternal *HFE* genotype related to human hemochromatosis and transferrin (TF) genotypes [41], mercury levels and maternal *GSTM1* and *GSTT1* genotypes [42], iron levels and maternal *GSTM1* genotypes [43], organochlorine pesticide levels and maternal *GSTM1* and *CYP17A1* genotypes [44, 45], perfluoroalkyl substance levels and maternal *GSTM1* genotypes [46], dioxin levels and maternal *AHR* and *GSTM1* genotypes [47, 57], $PM_{2.5}$ levels and fetal *CYP2D6* and *GSTP1* genotypes [35], benzo(a)pyrene levels and fetal *GSTP1* genotypes [36], and the association between alcohol consumption and fetal *ADH2* genotype [50]. Increased risk of LBW is affected by the association between floriculture chemicals and maternal paraoxonase 1 (*PON1*) genotype [52], disinfection by-product levels in drinking water and maternal *GSTM1* genotype [54], and between phthalate levels and fetal paraoxonase 2 (*PON2*) genotypes [52]. Increased risk of SGA is affected by the association between disinfection by-product levels in drinking water and maternal *CYP2E1* and *GSTT1* genotypes [55], and that between disinfection by-product levels in drinking water and fetal *CYP17A1* genotypes [58]. No risk of SGA is affected by the association between caffeine levels and maternal and infant *CYP1A2* genotypes, and the association between caffeine levels and maternal and infant *CYP2E1* genotypes [59]. Reduced gestational age is affected by the association between NO_x levels and maternal interleukin *(IL)-17A* genotype [48], and the association between alkyl phosphate levels and maternal *PON1* genotypes [49]. Increased risk of IUGR is affected by the association between alcohol consumption and maternal *CYP17* genotypes [55], and that between disinfection by-product levels of drinking water and fetal *CYP2E1* genotype [56].

Many maternal and infant genes related to chemical substances have never been examined in relation to those involved in xenobiotic metabolism and hormone biosynthesis. However, at present, epidemiological evidence of disease susceptibility genes is limited with regard to gene–environment interactions for maternal chemical exposure and infant growth.

Additional studies are required to examine not only the genotypes of exposure susceptibility genes encoding metabolizing enzymes and receptors related to extraneous substances, but also genetic polymorphisms of disease susceptibility genes such as growth- and obesity-related genes. The results from epidemiological studies on the effects of gene–environment interactions on infant growth may also be of value for planning environmental policies involving genetically high-risk groups and public health programs, as well as for preventive medicine.

19.4 Recent Advances in Genome Birth Cohort Studies and Evaluation of Genetic Associations with Birth Weight

In large-scale epidemiological studies such as genome birth cohort studies, evaluation of gene–environment interactions is different from that in small-scale epidemiological studies (described in Sects. 19.2 and 19.3). The main reason for this

difference is that, unlike hundreds of thousands of genome and epigenome data, it is difficult to obtain environmental data for each subject, except for their cigarette smoking or alcohol intake status. The development of strategies to address this difference in data availability is one of the most important challenges in large-scale epidemiological studies. Therefore, various methods for analyzing genetic susceptibility, such as GWAS, Mendelian randomization, and exposome linked with genomics, have been implemented.

19.4.1 GWAS and Meta-Analyses

A GWAS implements a population-based experimental design to detect associations between genetic variants and diseases or traits in biological samples from various human genome cohorts [7]. Meta-analyses of GWAS have identified numerous genetic variants associated with birth weight [60–72] (Table 19.3). Two SNPs, rs900400 near *CCNL1* and rs9883204 in *ADCY5*, were identified to be robustly associated with birth weight [60]. Another related SNP in *ADCY5* is considered to be implicated in the regulation of glucose levels and susceptibility to type 2 diabetes based on findings of an adult GWAS [73]. This may be the first evidence that associations between lower birth weight and subsequent non-communicable diseases (NCDs) such as type 2 diabetes have a genetic component; this is known as the developmental origins of health and disease (DOHaD) concept. Furthermore, an expanded GWAS meta-analysis and follow-up study involving 69,308 individuals of European descent from 43 studies revealed seven loci (*CCNL1*, *ADCY5*, *HMGA2*, *CDKAL1*, *LCORL*, *ADRB1*, and *5q11.2*) associated with birth weight with genome-wide significance [66]. Among them, five loci are known to be associated with other adult phenotypes: *ADCY5* and *CDKAL1* with type 2 diabetes, *ADRB1* with blood pressure, and *HMGA2* and *LCORL* with height. These findings highlight multiple genetic links between birth weight and postnatal growth and metabolism, especially later in life, which are important in accordance with the DOHaD concept from a genetic point of view. Moreover, a multi-ancestry GWAS meta-analysis of birth weight in 153,781 individuals from the EGG Consortium and the UK Biobank identified 60 loci where fetal genotype was associated with birth weight with genome-wide significance [70]. This study revealed strong inverse genetic correlations between birth weight and adult cardiometabolic diseases and traits such as type 2 diabetes and coronary artery disease, blood pressure, cholesterol levels, and triglyceride levels (Fig. 19.2). Thus, a series of GWAS meta-analyses have confirmed genetic involvement in life-course associations between early growth phenotypes and adult cardiometabolic diseases and traits. GWASs of birth weight have thus far focused on fetal genetics, whereas relatively little is known about the role of maternal genetic variation. Recently, similar GWAS meta-analyses in up to 86,577 women of European descent from the same population revealed maternal loci associated with offspring's birth weight, such as *MTNR1B*, *HMGA2*, *SH2B3*, *KCNAB1*, *L3MBTL3*, *GCK*, *EBF1*, *TCF7L2*, *ACTL9*, and *CYP3A7*, at GWAS significance [71]. Interestingly, maternal genetic factors associated with glucose metabolism,

Table 19.3 Genome-wide association studies (GWASs) and meta-analyses

Subjects	SNPs	Environmental factors	Outcomes	Results	Diseases/traits related SNPs	References
6 EGG birth cohort studies (n = 10,623) 13 EGG replication studies (n = 27,591)	rs900400 near *CCNL1* rs9883204 in *ADCY5*	Smoking: n.s.	Birth weight Birth weight	$\beta = -0.086$ ($p = 2 \times 10^{-35}$) $\beta = -0.063$ ($p = 7 \times 10^{-15}$)	– Type 2 diabetes	Freathy et al. [60]
Population-based Inter99 study (n = 4,744) Meta-analysis (n = 25,164)	rs11708067 in *ADCY5* rs7756992 in *CDKAL1* rs1111875 in *HHEX-IDE*	n.d.	Birth weight Birth weight Birth weight	$\beta = -33$ ($p = 0.004$) $\beta = -22$ ($p = 0.04$) $\beta = -16$ (8×10^{-5})	Type 2 diabetes Type 2 diabetes Type 2 diabetes	Andersson et al. [61]
Population-based Inter99 study (n = 4,744) Meta-analysis (n = 5484 Danish and 6915 Finnish non-diabetic individuals)	rs900400 near *CCNL1*	n.d.	Birth weight	$\beta = -22.1$ ($p = 0.024$)	Insulinogenic index Disposition index	Andersson et al. [62]
3 adult cohorts (n = 14,060) 6 GWAS for birth weight (n = 10,623) Published data (n = 14,837)	rs10838738 in *MTCH2* rs1121980 in *FTO*	n.d.	Birth weight Birth weight	$\beta = -13$ ($p = 0.012$) $\beta = 11$ ($p = 0.013$)	BMI BMI	Kilpeläinen et al. [63]
393 families from the US 265 families from Argentina 735 mother-infant pairs from Denmark	rs9883204 in *ADCY5* rs900400 near *CCNL1*	Smoking: the effect was slightly more pronounced ($p = 0.036$)	Birth weight Birth weight	n.s. $\beta = -0.069$ ($p = 0.068$)	Type 2 diabetes –	Ryckman et al. [64]
4 ethnic groups (n = 4,281) Meta-analysis (n = 2,296)	rs900400 near *CCNL1*	Smoking: adjusted	Newborn adiposity	Z score = 7.356 ($P = 1.90 \times 10^{-13}$)	BMI	Urbanek et al. [65]

43 EGG birth cohort studies (n = 69,308 individuals of European descent)	rs900400 near *CCNL1* rs9883204 in *ADCY5* rs6931514 in *CDKAL1* rs1042725 in *HMGA2* rs724577 in *LCORL* rs1801253 in *ADRB1* rs4432842 at *5q11.2*	Smoking	Birth weight Birth weight Birth weight Birth weight Birth weight Birth weight	$\beta = -0.072$ ($p = 3.6 \times 10^{-38}$) $\beta = -0.059$ ($p = 5.5 \times 10^{-20}$) $\beta = -0.050$ ($p = 1.5 \times 10^{-18}$) $\beta = -0.047$ ($p = 1.4 \times 10^{-19}$) $\beta = -0.042$ ($P = 4.6 \times 10^{-11}$) $\beta = -0.041$ ($P = 3.6 \times 10^{-9}$) $\beta = -0.034$ ($P = 4.6 \times 10^{-8}$)	– Type 2 diabetesType 2 diabetes Height Height Blood pressure –	Horikoshi et al. [66]
GWAS: TwinsUK cohort (n = 4,593 females) Replication: Australian twin cohort (n = 3003), and UK-based singleton-birth individuals from the Hertfordshire cohort (n = 2,997)	rs12340987 and rs7849941 near *NTRK2*	Smoking	Birth weight	$\beta = -0.13$ ($p = 1.48 \times 10^{-8}$) $\beta = -0.12$ ($p = 5.46 \times 10^{-8}$)	Obesity	Metrustry et al. [67]
4 birth cohort studies in England, France, and Spain (n = 3031)	The obesity risk-allele score	n.d.	Infant size at birth Weight at age 1 year Length at age 1 year BMI at age 1 year Weight at age 2–3 years Height at age 2–3 years BMI at age 2–3 years	n.s. $\beta = 0.020$ ($p = 0.009$) $\beta = 0.020$ ($p = 0.01$) n.s. $\beta = 0.033$ ($P < 0.001$) $\beta = 0.025$ ($P < 0.001$) $\beta = 0.024$ ($P = 0.003$)	Obesity	Elks et al. [68]

(continued)

Table 19.3 (continued)

Subjects	SNPs	Environmental factors	Outcomes	Results	Diseases/traits related SNPs	References
Individuals with type 2 diabetes ($n = 3627$) and control participants (12,974) of European ancestry from the Nurses' Health Study and the Health Professionals Follow-Up Study	The genetic risk score (GRS): five low-birth weight-related SNPs	Smoking: adjusted	Birth weight	$\beta = -0.014$ ($p = 0.001$, women) $\beta = -0.018$ ($p = 0.001$, men)	Type 2 diabetes	Wang et al. [69]
40 EGG birth cohorts ($n = 75,891$ individuals of European descent) UK Biobank ($n = 67,786$ individuals of European descent) 6 EGG birth cohorts (10,104 individuals of non-European descent)	60 loci (Fig. 19.2)	Smoking $p < 0.015$	Birth weight	Both $p < 5 \times 10^{-8}$	Adult cardiometabolic diseases and traits (type 2 diabetes, fasting glucose, coronary artery disease, systolic and diastolic blood pressure, total, LDL and HDL cholesterol, triglycerides, adult height, BMI, etc.)	Horikoshi et al. [70]
EGG birth cohorts ($n = 37,945$ individuals of European descent) UK Biobank ($n = 48,632$ individuals of European descent)	Maternal SNPs at 10 loci (*MTNR1B, HMGA2, SH2B3, KCNAB1, L3MBTL3, GCK, EBF1, TCF7L2, ACTL9, CYP3A7*)	Smoking $p < 0.05$	Birth weight	Both $p < 5 \times 10^{-8}$	MTNR1B, GCK, and TCF7L2: fasting glucose CYP3A7: sex hormone levels EBF1: gestational duration	Beaumont et al. [71]
EGG birth cohorts and UK Biobank ($n = 321,223$ children and 230,069 mothers)	72 loci	n.d.	Birth weight	Both $p < 6.6 \times 10^{-9}$	Future cardio-metabolic health outcomes (the inverse birth weight-blood pressure association is attributable to genetic effects)	Warrington et al. [72]

n.d. not determined, *n.s.* not significant

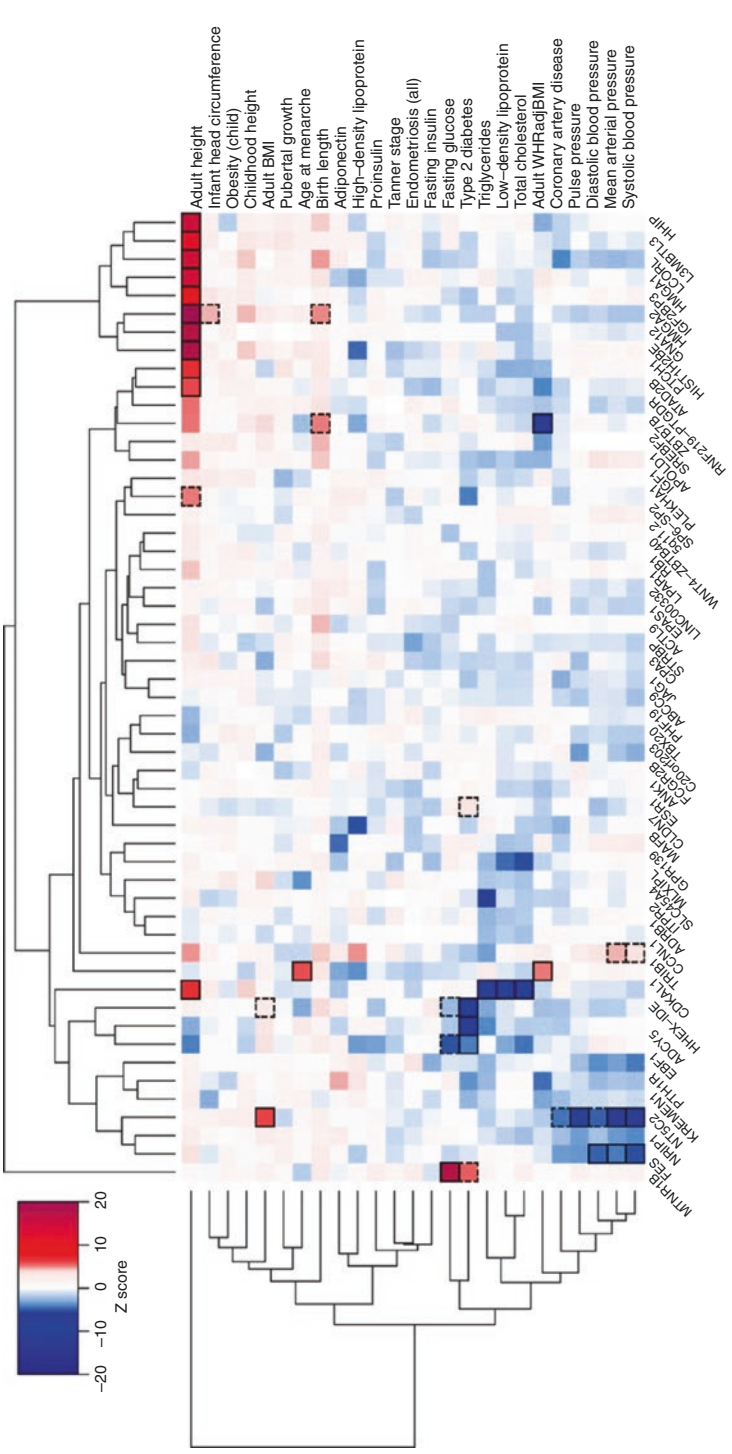


Fig. 19.2 Hierarchical clustering of birth weight (BW) loci based on the similarity of overlap with adult diseases, and metabolic and anthropometric traits [70]. "For the lead SNP at each BW locus (*x* axis), *Z* scores (aligned to BW-raising allele) were obtained from publicly available GWAS for various traits (*y* axis). A positive *Z* score (red) indicates a positive association between the BW-raising allele and the outcome trait, whereas a negative *Z* score (blue) indicates an inverse association. BW loci and traits were clustered according to the Euclidean distance among *Z* scores. Squares are outlined with a solid black line if the BW locus is significantly ($P < 5 \times 10^{-8}$) associated with the trait in publicly available GWAS, or with a dashed line if reported significant elsewhere" [70].

blood pressure, immune function, cytochrome P450 activity, and gestational duration affect the offspring's birth weight. In a recent study, expanded GWASs of the same population examining maternal (n = 321,223) and offspring birth weight (n = 230,069 mothers) revealed 190 independent loci associated with both the mother and offspring's birth weight [72]. In this study, structural equation modeling was used to evaluate the contribution of direct fetal and indirect maternal genetic effects, and Mendelian randomization was applied to reveal causal pathways. Surprisingly, maternal birth weight-lowering genotypes as proxy for an adverse intrauterine environment were found not to affect offspring blood pressure at all [72]. This finding indicates that some exceptions cannot be explained by the DOHaD concept.

There are few GWAS meta-analyses that have examined the influence of smoking on birth weight; therefore, it is necessary to systematically evaluate gene–environment interactions in relation to smoking, especially genome-wide gene-smoking interactions. Notably, genome-wide gene-smoking interaction studies have become more common in adult GWAS meta-analyses to identify new loci associated with adult traits such as obesity, blood pressure, and serum lipids [74–76].

19.4.2 Mendelian Randomization

An alternative method, Mendelian randomization, entails the use of genetic variants as proxies for the environmental exposure under investigation [77]. Mendelian randomization is a GWAS-based theoretical method for environmental risk assessment that uses genetic factors associated with environmental factors to assess the causal effect on internal biomarkers such as body mass index (BMI), systolic blood pressure, and fasting glucose levels [8, 78]. Birth weight was used as both the outcome of maternal internal biomarkers and the internal marker of fetal intrauterine environment [78–86] (Table 19.4). Until recently, genetic risk scores were preferred over SNPs in Mendelian randomization studies [68, 78, 82, 83]. Novel approaches to obtaining genetic risk scores include assessments of the genetic contribution of certain intermediate traits or risk factors to cardiometabolic disease, risk prediction in high-risk populations, studies of gene–environment interactions, and Mendelian randomization [87].

Mendelian randomization is a recent developing field that involves the use of genetic variation to assess the causal relationship between exposure and outcome, where genetic variants within or near coding loci related to protein concentrations enable assessment of their causal role in disease. However, the more complex relationship between genetic variation and exposure makes the findings from Mendelian randomization more difficult to interpret [87]. Recently, the use of maternal birth weight-lowering genotypes to proxy for an adverse intrauterine environment in Mendelian randomization analyses yielded no evidence that such genotype causally raises offspring blood pressure [72]. Mendelian randomization is considered an established method for strengthening causal inference and estimating causal effects; however, the use of genetic instruments, which lack direct links to measurement in most cases, may be one of the limitations of Mendelian randomization.

Table 19.4 Mendelian randomization

Design	Instrumental variables	Expected environmental factors	Outcomes	Results	References
Hospital-based study ($n = 473$)	*MTHFR* C677T (rs1801133)	Homocysteine	Birth weight	Homocysteine: marginal significance ($p = 0.06$)	Lee et al. [79]
Pune Maternal Nutrition Study (PMNS) ($n = 526$) and Parthenon Cohort Study ($n = 517$)	*MTHFR* C677T (rs1801133)	Homocysteine	Birth weight	Maternal homocysteine concentration (meta-analysis): β (95% CI) = −40 g (−62, −17) Maternal risk genotype at rs1801133 (meta-analysis): β (95% CI) = −61 g (−111, −10)	Yajnik et al. [80]
A population-based prospective birth cohort study ($n = 3, 778$)	*TAS2R38* genotype (rs713598)	6-*n*-propylthiouracil (PROP) taster status	BMI	BMI (SD score): $\beta = -0.09$ ($p = 0.023$, girls) Body fat mass (%): $\beta = -0.49$ ($p = 0.028$, girls)	Bouthoorn et al. [81]
Birth cohorts collected from three Nordic countries (Finland, Denmark, and Norway) ($n = 3,485$ mother/infant pairs)	A genetic score based on 697 SNPs known to be associated with adult height to index maternal height	Adult height	Birth weight, birth length, and gestational age	Maternal height was significantly associated with birth length ($p = 6.31 \times 10^{-9}$), birth weight ($p = 2.19 \times 10^{-15}$), and gestational age ($p = 1.51 \times 10^{-7}$).	Zhang et al. [82]
18 EGG birth cohorts ($n = 30,487$ women of European ancestry)	Genetic scores for BMI, fasting glucose level, type 2 diabetes, systolic blood pressure (SBP), triglyceride level, high-density lipoprotein cholesterol (HDL-C) level, vitamin D status, and adiponectin level	BMI, fasting glucose level, type 2 diabetes, systolic blood pressure (SBP), triglyceride level, high-density lipoprotein cholesterol (HDL-C) level, vitamin D status, and adiponectin level	Birth weight	The maternal genetic score for BMI: β (95% CI) = 2 g (0–3 g; $p = 0.008$) Fasting glucose: b (95% CI) = 8 g (6–10 g; $p = 7 \times 10^{-14}$) SBP: β (95% CI) = −4 g (−6 to −2 g; $p = 1 \times 10^{-5}$)	Tyrrell et al. [78]

(continued)

Table 19.4 (continued)

Design	Instrumental variables	Expected environmental factors	Outcomes	Results	References
The Nurses' Health Study and the Health Professionals Follow-Up Study (n = 3627 individuals with type 2 diabetes and 12,974 control participants of European ancestry)	5 low-birthweight-related SNPs A genetic risk score (GRS) was calculated based on them	Birth weight	Type 2 diabetes	OR (95% CI) = 2.94 (1.70, 5.16; $p < 0.001$)	Wang et al. [83]
The CARDIoGRAMplusC4D 1000 genomes based GWAS case (n = 60,801)-control (n = 123,504) study and on lipids using GLGC (n = 188,577)	7 SNPs independently contributing to birth weight at genome-wide significance ($p < 5 \times 10^{-8}$)	Birth weight	IHD HDL cholesterol	IHD: OR (95% CI) = 0.96/100 g (0.93–0.99) HDL cholesterol: OR (95% CI) = −0.014 SD (−0.027 to −0.0005)	Au Yeung et al. [84]
The GUSTO study (n = 898 mothers and 1103 offspring)	35 SNPs in *FADS1, FADS2*, and *FADS3*	Maternal plasma n-3 and n-6 PUFA concentrations	Gestation duration	rs174450 minor allele C: β (95% CI) = 2.2 day (0.9, 3.4 day) for maternal variants; 1.9 day (0.7, 3.0 day) for offspring variants	Bernard et al. [85]
GIANT consortium (n = 224,459 individuals of European ancestry)	41 SNPs: hip circumference adjusted for BMI	Maternal central obesity	Birth weight	Hip circumference (HIP) was associated with a 0.392 SD increase in birth length ($p = 1.1 \times 10^{-6}$) and a 0.168 SD increase in birth weight ($p = 7.1 \times 10^{-5}$)	Geng et al. [86]

OR odds ratio, *CI* confidence interval, *SD* standard deviation

19.4.3 Exposome Linked with Genomics

Although GWASs have revealed genetic associations and networks that improve the understanding of diseases, these findings only elucidate a small part of overall disease risk [88]. As disease causation is largely non-genetic, the need for improved tools to quantify environmental contributions seems obvious [89]. The "exposome" was originally defined as representing all kinds of environmental exposure, including those from diet, lifestyle, and endogenous sources, during the entire lifespan from the prenatal period onward, as a quantity of critical interest to disease etiology [90]. Three overlapping domains within the exposome have been described as follows: (1) a general external environment to include factors such as the urban environment, climate factors, social capital, and stress; (2) a specific external environment with specific contaminants, diet, physical activity, tobacco, and infections; and (3) an internal environment to include internal biological factors such as metabolic factors, gut microflora, inflammation, and oxidative stress [91]. Although it is difficult to elucidate the exposome entirely at present, the inherent value of exposomic data in cohort studies is that they can provide a greater understanding of relationships between a wide range of exposure and health conditions, and ultimately lead to more effective and efficient disease prevention and control [92]. For example, the NIH Child Health Environmental Analysis Resource (CHEAR) is a major step toward providing the infrastructure needed to study the exposome in relation to child health [9]. Furthermore, an EU-funded project, EXPOsOMICS, aims to develop a novel approach for assessing exposure to high-priority environmental pollutants, such as air and water contaminants, during critical periods of life, by characterizing the external and internal components of the exposome [93, 94]. Exposome research in the context of developing interventions is targeted at the population level to improve public health, whereas the application of genomics lies in preventive and therapeutic interventions targeted at individuals [94].

19.4.4 Perspectives

Analysis of genetic factors and environmental exposure offers evidence-based explanations for the associations between LBW, early growth, and increased propensity to develop NCDs in later life [60, 66, 70–72, 95]. Among epidemiological studies, GWASs have revealed the relative role of genes and the environment in disease risk, assisting in risk prediction such as in preemptive and precision medicine [96, 97]. Although Mendelian randomization is a powerful tool that utilizes genetic information to explain the likely causal relevance of an exposure to an outcome and increasingly complex gene-to-exposure and exposure-to-outcome relationships; thus, it is more difficult to perform reliable conduct and interpretation [8]. Tremendous strides are being achieved toward developing an exposomics approach together with infrastructural progress toward the identification of new methods and consortia that can address big-picture questions of how environment impacts health

and development [9, 93]. Future studies should aim to develop new methods and analytical approaches for exposure assessment and data harmonization [9].

In Japan, the concept of preemptive medicine, which is a novel medical paradigm that advocates for pre-symptomatic diagnosis or prevention intervention at an early stage to prevent disease onset, has been proposed, and policy strategic proposals based on this approach have been made [97, 98]. Recently, collaborations of birth cohort studies are being supported by the project for babies and infants in research of health and development to adolescent and young adult (BIRTHDAY) of the Japan Agency for Medical Research and Development (AMED) on the basis of the current health policy "Overcoming health issues according to life stage" [99]. The rapid progress of large-scale epidemiological studies for elucidating gene–environment interactions is expected to occur in Japan in the near future.

19.5 Conclusions

Analysis of gene–environment interactions between tobacco and environmental chemicals and candidate genes, such as those encoding metabolic enzymes including CYPs and GSTs, has been performed via small-scale epidemiological studies. In the last 10 years, major innovations in research designs and methods, accompanied by recent rapid advances of analytical and measurement technologies, have occurred. In particular, GWASs and EWASs have become mainstream in genome cohort studies using advanced genomics and epigenomics, which has made it possible to better understand the genetic basis of diseases. As disease causation is largely non-genetic, the concept of the exposome was proposed as an improved tool to quantify total environmental contributions. Linking the exposome with genomics, in combination with preemptive and precision medicine, is expected to reveal novel insights into the origins of multiple complex diseases.

Acknowledgements Our work was supported in part by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science; the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18gk0110032.

References

1. Kishi R, Sata F, Yoshioka E, Ban S, Sasaki S, Konishi K, et al. Exploiting gene-environment interaction to detect adverse health effects of environmental chemicals on the next generation. Basic Clin Pharmacol Toxicol. 2008;102(2):191–203. https://doi.org/10.1111/j.1742-7843.2007.00201.x.
2. Kelada SN, Eaton DL, Wang SS, Rothman NR, Khoury MJ. The role of genetic polymorphisms in environmental health. Environ Health Perspect. 2003;111(8):1055–64. https://doi.org/10.1289/ehp.6065.

3. Edwards TM, Myers JP. Environmental exposures and gene regulation in disease etiology. Environ Health Perspect. 2007;115(9):1264–70. https://doi.org/10.1289/ehp.9951.
4. Kraft P, Hunter D. Integrating epidemiology and genetic association: the challenge of gene-environment interaction. Philos Trans R Soc Lond B Biol Sci. 2005;360(1460):1609–16. https://doi.org/10.1098/rstb.2005.1692.
5. Kishi R, Sasaki S, Yoshioka E, Yuasa M, Sata F, Saijo Y, et al. Cohort profile: The Hokkaido Study on Environment and Children's Health in Japan. Int J Epidemiol. 2011;40(3):611–8. https://doi.org/10.1093/ije/dyq071.
6. Kishi R, Kobayashi S, Ikeno T, Araki A, Miyashita C, Itoh S, et al. Ten years of progress in The Hokkaido Birth Cohort Study on Environment and Children's Health: cohort profile--updated 2013. Environ Health Prev Med. 2013;18(6):429–50. https://doi.org/10.1007/s12199-013-0357-3.
7. Visscher PM, Wray NR, Zhang Q, Sklar P, McCarthy MI, Brown MA, et al. 10 years of GWAS discovery: biology, function, and translation. Am J Hum Genet. 2017;101(1):5–22. https://doi.org/10.1016/j.ajhg.2017.06.005.
8. Holmes MV, Ala-Korpela M, Smith GD. Mendelian randomization in cardiometabolic disease: challenges in evaluating causality. Nat Rev Cardiol. 2017;14(10):577–90. https://doi.org/10.1038/nrcardio.2017.78.
9. Wright RO. Environment, susceptibility windows, development, and child health. Curr Opin Pediatr. 2017;29(2):211–7. https://doi.org/10.1097/MOP.0000000000000465.
10. Smith MT, de la Rosa R, Daniels SI. Using exposomics to assess cumulative risks and promote health. Environ Mol Mutagen. 2015;56(9):715–23. https://doi.org/10.1002/em.21985.
11. Wang X, Zuckerman B, Pearson C, Kaufman G, Chen C, Wang G, et al. Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. JAMA. 2002;287(2):195–202. https://doi.org/10.1001/jama.287.2.195.
12. Sasaki S, Kondo T, Sata F, Saijo Y, Katoh S, Nakajima S, et al. Maternal smoking during pregnancy and genetic polymorphisms in the Ah receptor, CYP1A1 and GSTM1 affect infant birth size in Japanese subjects. Mol Hum Reprod. 2006;12(2):77–83. https://doi.org/10.1093/molehr/gal013.
13. Sasaki S, Sata F, Katoh S, Saijo Y, Nakajima S, Washino N, et al. Adverse birth outcomes associated with maternal smoking and polymorphisms in the N-Nitrosamine-metabolizing enzyme genes NQO1 and CYP2E1. Am J Epidemiol. 2008;167(6):719–26. https://doi.org/10.1093/aje/kwm360.
14. Gloria-Bottini F, Magrini A, Cozzoli E, Bergamaschi A, Bottini E. ADA genetic polymorphism and the effect of smoking on neonatal bilirubinemia and developmental parameters. Early Hum Dev. 2008;84(11):739–43. https://doi.org/10.1016/j.earlhumdev.2008.05.001.
15. Grazuleviciene R, Nieuwenhuijsen MJ, Danileviciute A, Nadisauskiene R, Buinauskiene J. Gene-environment interaction: maternal smoking and contribution of GSTT1 and GSTM1 polymorphisms to infant birth-weight reduction in a Kaunas Cohort Study. J Epidemiol Community Health. 2010;64(7):648. https://doi.org/10.1136/jech.2009.100859.
16. Aagaard-Tillery K, Spong CY, Thom E, Sibai B, Wendel G Jr, Wenstrom K, et al. Pharmacogenomics of maternal tobacco use: metabolic gene polymorphisms and risk of adverse pregnancy outcomes. Obstet Gynecol. 2010;115(3):568–77. https://doi.org/10.1097/AOG.0b013e3181d06faf.
17. Karahalil B, Emerce E, Kocabaş NA, Akkaş E. Associations between GSTM1 and OGG1 Ser326Cys polymorphisms and smoking on chromosomal damage and birth growth in mothers. Mol Biol Rep. 2011;38(5):2911–8. https://doi.org/10.1007/s11033-010-9953-0.
18. Danileviciute A, Grazuleviciene R, Paulauskas A, Nadisauskiene R, Nieuwenhuijsen MJ. Low level maternal smoking and infant birthweight reduction: genetic contributions of GSTT1 and GSTM1 polymorphisms. BMC Pregnancy Childbirth. 2012;12:161. https://doi.org/10.1186/1471-2393-12-161.
19. Zheng X, Feingold E, Ryckman KK, Shaffer JR, Boyd HA, Feenstra B, et al. Association of maternal CNVs in GSTT1/GSTT2 with smoking, preterm delivery, and low birth weight. Front Genet. 2013;4:196. https://doi.org/10.3389/fgene.2013.00196.

20. Yila TA, Sasaki S, Miyashita C, Braimoh TS, Kashino I, Kobayashi S, et al. Effects of maternal 5,10-methylenetetrahydrofolate reductase C677T and A1298C Polymorphisms and tobacco smoking on infant birth weight in a Japanese population. J Epidemiol. 2012;22(2):91–102. https://doi.org/10.2188/jea.JE20110039.
21. Kobayashi S, Sata F, Sasaki S, Braimoh TS, Araki A, Miyashita C, et al. Combined effects of AHR, CYP1A1, and XRCC1 genotypes and prenatal maternal smoking on infant birth size: biomarker assessment in the Hokkaido Study. Reprod Toxicol. 2016;65:295–306. https://doi.org/10.1016/j.reprotox.2016.08.020.
22. Hong YC, Lee KH, Son BK, Ha EH, Moon HS, Ha M. Effects of the GSTM1 and GSTT1 polymorphisms on the relationship between maternal exposure to environmental tobacco smoke and neonatal birth weight. J Occup Environ Med. 2003;45(5):492–8. https://doi.org/10.1097/01.jom.0000063627.37065.a1.
23. Wu T, Hu Y, Chen C, Yang F, Li Z, Fang Z, et al. Passive smoking, metabolic gene polymorphisms, and infant birth weight in a prospective cohort study of Chinese women. Am J Epidemiol. 2007;166(3):313–22. https://doi.org/10.1093/aje/kwm090.
24. Kobayashi S, Sata F, Sasaki S, Braimoh TS, Araki A, Miyashita C, Goudarzi H, et al. Modification of adverse health effects of maternal active and passive smoking by genetic susceptibility: dose-dependent association of plasma cotinine with infant birth size among Japanese women-The Hokkaido Study. Reprod Toxicol. 2017;74:94–103. https://doi.org/10.1016/j.reprotox.2017.09.002.
25. Infante-Rivard C, Weinberg CR, Guiguet M. Xenobiotic-metabolizing genes and small-for-gestational-age births: interaction with maternal smoking. Epidemiology. 2006;17(1):38–46. https://doi.org/10.1097/01.ede.0000187669.34003.b1.
26. Xie C, Wen X, Niu Z, Ding P, Liu T, He Y, et al. Combinations of CYP2A6∗4 and glutathione S-transferases gene polymorphisms modify the association between maternal second-hand smoke exposure during pregnancy and small-for-gestational-age. Nicotine Tob Res. 2015;17(12):1421–7. https://doi.org/10.1093/ntr/ntv072.
27. Huang KH, Chou AK, Jeng SF, Ng S, Hsieh CJ, Chen MH, Chen PC, Hsieh WS. The impacts of cord blood cotinine and glutathione-S-transferase gene polymorphisms on birth outcome. Pediatr Neonatol. 2017;58(4):362–9. https://doi.org/10.1016/j.pedneo.2016.08.006.
28. Delpisheh A, Brabin L, Topping J, Reyad M, Tang AW, Brabin BJ. A case-control study of CYP1A1, GSTT1 and GSTM1 gene polymorphisms, pregnancy smoking and fetal growth restriction. Eur J Obstet Gynecol Reprod Biol. 2009;143(1):38–42. https://doi.org/10.1016/j.ejogrb.2008.11.006.
29. Price TS, Grosser T, Plomin R, Jaffee SR. Fetal genotype for the xenobiotic metabolizing enzyme NQO1 influences intrauterine growth among infants whose mothers smoked during pregnancy. Child Dev. 2010;81(1):101–14. https://doi.org/10.1111/j.1467-8624.2009.01383.x.
30. Nukui T, Day RD, Sims CS, Ness RB, Romkes M. Maternal/newborn GSTT1 null genotype contributes to risk of preterm, low birthweight infants. Pharmacogenetics. 2004;14(9):569–76.
31. Tsai HJ, Liu X, Mestan K, Yu Y, Zhang S, Fang Y, et al. Maternal cigarette smoking, metabolic gene polymorphisms, and preterm delivery: new insights on GxE interactions and pathogenic pathways. Hum Genet. 2008;123(4):359–69. https://doi.org/10.1007/s00439-008-0485-9.
32. Huang H, Clancy KB, Burhance C, Zhu Y, Madrigal L. Women who deliver twins are more likely to smoke and have high frequencies of specific SNPs: results from a sample of African-American women who delivered preterm, low birth weight babies. Am J Hum Biol. 2015;27(5):605–12. https://doi.org/10.1002/ajhb.22723.
33. Grazuleviciene R, Danileviciute A, Nadisauskiene R, Vencloviene J. Maternal smoking, GSTM1 and GSTT1 polymorphism and susceptibility to adverse pregnancy outcomes. Int J Environ Res Public Health. 2009;6(3):1282–97. https://doi.org/10.3390/ijerph6031282.
34. Suh YJ, Kim BM, Park BH, Park H, Kim YJ, Kim H, et al. Cytochrome P450IA1 polymorphisms along with PM(10) exposure contribute to the risk of birth weight reduction. Reprod Toxicol. 2007;24(3-4):281–8. https://doi.org/10.1016/j.reprotox.2007.07.001.
35. Slama R, Gräbsch C, Lepeule J, Siroux V, Cyrys J, Sausenthaler S, et al. Maternal fine particulate matter exposure, polymorphism in xenobiotic-metabolizing genes and offspring birth weight. Reprod Toxicol. 2010;30(4):600–12. https://doi.org/10.1016/j.reprotox.2010.07.001.

36. Duarte-Salles T, Mendez MA, Morales E, Bustamante M, Rodríguez-Vicente A, Kogevinas M, et al. Dietary benzo(a)pyrene and fetal growth: effect modification by vitamin C intake and glutathione S-transferase P1 polymorphism. Environ Int. 2012;45:1–8. https://doi.org/10.1016/j.envint.2012.04.002.
37. Sasaki S, Limpar M, Sata F, Kobayashi S, Kishi R. Interaction between maternal caffeine intake during pregnancy and CYP1A2 C164A polymorphism affects infant birth size in the Hokkaido Study. Pediatr Res. 2017;82(1):19–28. https://doi.org/10.1038/pr.2017.70.
38. Chun SK, Shin S, Kim MY, Joung H, Chung J. Effects of maternal genetic polymorphisms in vitamin D-binding protein and serum 25-hydroxyvitamin D concentration on infant birth weight. Nutrition. 2017;35:36–42. https://doi.org/10.1016/j.nut.2016.10.006.
39. Ruiz JR, Labayen I, Ortega FB, Moreno LA, González-Lamuño D, Martí A, et al. Birth weight and blood lipid levels in Spanish adolescents: influence of selected APOE, APOC3 and PPARgamma2 gene polymorphisms. The AVENA Study. BMC Med Genet. 2008;9:98. https://doi.org/10.1186/1471-2350-9-98.
40. Moltó-Puigmartí C, van Dongen MC, Dagnelie PC, Plat J, Mensink RP, Tan FE, et al. Maternal but not fetal FADS gene variants modify the association between maternal long-chain PUFA intake in pregnancy and birth weight. J Nutr. 2014;144(9):1430–7. https://doi.org/10.3945/jn.114.194035.
41. Cantonwine D, Hu H, Téllez-Rojo MM, Sánchez BN, Lamadrid-Figueroa H, Ettinger AS, et al. HFE gene variants modify the association between maternal lead burden and infant birthweight: a prospective birth cohort study in Mexico City, Mexico. Environ Health. 2010;9:43. https://doi.org/10.1186/1476-069X-9-43.
42. Lee BE, Hong YC, Park H, Ha M, Koo BS, Chang N, et al. Interaction between GSTM1/GSTT1 polymorphism and blood mercury on birth weight. Environ Health Perspect. 2010;118(3):437–43. https://doi.org/10.1289/ehp.0900731.
43. Hur J, Kim H, Ha EH, Park H, Ha M, Kim Y, et al. Birth weight of Korean infants is affected by the interaction of maternal iron intake and GSTM1 polymorphism. J Nutr. 2013;143(1):67–73. https://doi.org/10.3945/jn.112.161638.
44. Sharma E, Mustafa M, Pathak R, Guleria K, Ahmed RS, Vaid NB, et al. A case control study of gene environmental interaction in fetal growth restriction with special reference to organochlorine pesticides. Eur J Obstet Gynecol Reprod Biol. 2012;161(2):163–9. https://doi.org/10.1016/j.ejogrb.2012.01.008.
45. Chand S, Mustafa MD, Banerjee BD, Guleria K. CYP17A1 gene polymorphisms and environmental exposure to organochlorine pesticides contribute to the risk of small for gestational age. Eur J Obstet Gynecol Reprod Biol. 2014;180:100–5. https://doi.org/10.1016/j.ejogrb.2014.06.016.
46. Kwon EJ, Shin JS, Kim BM, Shah-Kulkarni S, Park H, Kho YL, et al. Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. J Occup Environ Med. 2016;58(6):e198–205. https://doi.org/10.1097/JOM.0000000000000739.
47. Kobayashi S, Sata F, Miyashita C, Sasaki S, Ban S, Araki A, et al. Dioxin-metabolizing genes in relation to effects of prenatal dioxin levels and reduced birth size: The Hokkaido Study. Reprod Toxicol. 2017;67:111–6. https://doi.org/10.1016/j.reprotox.2016.12.002.
48. Nansook P, Naidoo RN, Muttoo S, Asharam K, Ramkaran P, Phulukdaree A, et al. IL-17A[G197G]-association between NOx and gestational age in a South African Birth Cohort. Int J Immunogenet. 2018;45(2):54–62. https://doi.org/10.1111/iji.12358.
49. Harley KG, Huen K, Aguilar Schall R, Holland NT, Bradman A, Barr DB, et al. Association of organophosphate pesticide exposure and paraoxonase with birth outcome in Mexican-American women. PLoS One. 2011;6(8):e23923. https://doi.org/10.1371/journal.pone.0023923.
50. Arfsten DP, Silbergeld EK, Loffredo CA. Fetal ADH2∗3, maternal alcohol consumption, and fetal growth. Int J Toxicol. 2004;23(1):47–54. https://doi.org/10.1080/10915810490265450.
51. Kogevinas M, Bustamante M, Gracia-Lavedán E, Ballester F, Cordier S, Costet N, et al. Drinking water disinfection by-products, genetic polymorphisms, and birth outcomes in a European Mother-Child Cohort Study. Epidemiology. 2016;27(6):903–11.
52. Moreno-Banda G, Blanco-Muñoz J, Lacasaña M, Rothenberg SJ, Aguilar-Garduño C, Gamboa R, et al. Maternal exposure to floricultural work during pregnancy, PON1 Q192R polymor-

phisms and the risk of low birth weight. Sci Total Environ. 2009;407(21):5478–85. https://doi.org/10.1016/j.scitotenv.2009.06.033.
53. Xie C, Jin R, Zhao Y, Lin L, Li L, Chen J, et al. Paraoxonase 2 gene polymorphisms and prenatal phthalates' exposure in Chinese newborns. Environ Res. 2015;140:354–9. https://doi.org/10.1016/j.envres.2015.03.028.
54. Danileviciute A, Grazuleviciene R, Vencloviene J, Paulauskas A, Nieuwenhuijsen MJ. Exposure to drinking water trihalomethanes and their association with low birth weight and small for gestational age in genetically susceptible women. Int J Environ Res Public Health. 2012;9(12):4470–85. https://doi.org/10.3390/ijerph9124470.
55. Delpisheh A, Topping J, Reyad M, Tang A, Brabin BJ. Prenatal alcohol exposure, CYP17 gene polymorphisms and fetal growth restriction. Eur J Obstet Gynecol Reprod Biol. 2008;138(1):49–53. https://doi.org/10.1016/j.ejogrb.2007.08.006.
56. Infante-Rivard C. Drinking water contaminants, gene polymorphisms, and fetal growth. Environ Health Perspect. 2004;112(11):1213–6. https://doi.org/10.1289/ehp.7003.
57. Ames J, Warner M, Mocarelli P, Brambilla P, Signorini S, Siracusa C, et al. AHR gene-dioxin interactions and birthweight in the Seveso Second Generation Health Study. Int J Epidemiol. 2018;47(6):1992–2004. https://doi.org/10.1093/ije/dyy165.
58. Bonou SG, Levallois P, Giguère Y, Rodriguez M, Bureau A. Prenatal exposure to drinking-water chlorination by-products, cytochrome P450 gene polymorphisms and small-for-gestational-age neonates. Reprod Toxicol. 2017;73:75–86. https://doi.org/10.1016/j.reprotox.2017.07.019.
59. Infante-Rivard C. Caffeine intake and small-for-gestational-age birth: modifying effects of xenobiotic-metabolising genes and smoking. Paediatr Perinat Epidemiol. 2007;21(4):300–9. https://doi.org/10.1111/j.1365-3016.2007.00825.x.
60. Freathy RM, Mook-Kanamori DO, Sovio U, Prokopenko I, Timpson NJ, Berry DJ, et al. Variants in ADCY5 and near CCNL1 are associated with fetal growth and birth weight. Nat Genet. 2010;42:430–5. https://doi.org/10.1038/ng.567.
61. Andersson EA, Pilgaard K, Pisinger C, Harder MN, Grarup N, Faerch K, et al. Type 2 diabetes risk alleles near ADCY5, CDKAL1 and HHEX-IDE are associated with reduced birthweight. Diabetologia. 2010;53(9):1908–16. https://doi.org/10.1007/s00125-010-1790-0.
62. Andersson EA, Harder MN, Pilgaard K, Pisinger C, Stančáková A, Kuusisto J, et al. The birth weight lowering C-allele of rs900400 near LEKR1 and CCNL1 associates with elevated insulin release following an oral glucose challenge. PLoS One. 2011;6(11):e27096. https://doi.org/10.1371/journal.pone.0027096.
63. Kilpeläinen TO, den Hoed M, Ong KK, Grøntved A, Brage S, Early Growth Genetics Consortium, et al. Obesity-susceptibility loci have a limited influence on birth weight: a meta-analysis of up to 28,219 individuals. Am J Clin Nutr. 2011;93(4):851–60. https://doi.org/10.3945/ajcn.110.000828.
64. Ryckman KK, Feenstra B, Shaffer JR, Bream EN, Geller F, Feingold E, et al. Replication of a genome-wide association study of birth weight in preterm neonates. J Pediatr. 2012;160(1):19–24. https://doi.org/10.1016/j.jpeds.2011.07.038.
65. Urbanek M, Hayes MG, Armstrong LL, Morrison J, Lowe LP, Badon SE, et al. The chromosome 3q25 genomic region is associated with measures of adiposity in newborns in a multiethnic genome-wide association study. Hum Mol Genet. 2013;22(17):3583–96. https://doi.org/10.1093/hmg/ddt168.
66. Horikoshi M, Yaghootkar H, Mook-Kanamori DO, Sovio U, Taal HR, Hennig BJ, et al. New loci associated with birth weight identify genetic links between intrauterine growth and adult height and metabolism. Nat Genet. 2013;45:76–82. https://doi.org/10.1038/ng.2477.
67. Metrustry SJ, Edwards MH, Medland SE, Holloway JW, Montgomery GW, Martin NG, et al. Variants close to NTRK2 gene are associated with birth weight in female twins. Twin Res Hum Genet. 2014;17(4):254–61. https://doi.org/10.1017/thg.2014.34.
68. Elks CE, Heude B, de Zegher F, Barton SJ, Clément K, Inskip HM, et al. Associations between genetic obesity susceptibility and early postnatal fat and lean mass: an individual participant meta-analysis. JAMA Pediatr. 2014;168(12):1122–30. https://doi.org/10.1001/jamapediatrics.2014.1619.

69. Wang T, Huang T, Li Y, Zheng Y, Manson JE, Hu FB, et al. Low birthweight and risk of type 2 diabetes: A Mendelian Randomisation Study. Diabetologia. 2016;59(9):1920–7. https://doi.org/10.1007/s00125-016-4019-z.
70. Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN, Fernandez-Tajes J, et al. Genome-wide associations for birth weight and correlations with adult disease. Nature. 2016;538:248–52.
71. Beaumont RN, Warrington NM, Cavadino A, Tyrrell J, Nodzenski M, Horikoshi M, et al. Genome-wide association study of offspring birth weight in 86,577 women identifies five novel loci and highlights maternal genetic effects that are independent of fetal genetics. Hum Mol Genet. 2018;27(4):742–56. https://doi.org/10.1093/hmg/ddx429.
72. Warrington NM, Beaumont RN, Horikoshi M, Day FR, Helgeland Ø, Laurin C, et al. Maternal and fetal genetic effects on birth weight and their relevance to cardio-metabolic risk factors. Nat Genet. 2019;51(5):804–14. https://doi.org/10.1038/s41588-019-0403-1.
73. Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010;42(2):105–16. https://doi.org/10.1038/ng.520.
74. Justice AE, Winkler TW, Feitosa MF, Graff M, Fisher VA, Young K, et al. Genome-wide meta-analysis of 241,258 adults accounting for smoking behaviour identifies novel loci for obesity traits. Nat Commun. 2017;8:14977. https://doi.org/10.1038/ncomms14977.
75. Sung YJ, Winkler TW, de Las Fuentes L, Bentley AR, Brown MR, Kraja AT, et al. A large-scale multi-ancestry genome-wide study accounting for smoking behavior identifies multiple significant loci for blood pressure. Am J Hum Genet. 2018;102(3):375–400. https://doi.org/10.1016/j.ajhg.2018.01.015.
76. Bentley AR, Sung YJ, Brown MR, Winkler TW, Kraja AT, Ntalla I, et al. Multi-ancestry genome-wide gene-smoking interaction study of 387,272 individuals identifies new loci associated with serum lipids. Nat Genet. 2019;51(4):636–48. https://doi.org/10.1038/s41588-019-0378-y.
77. Sleiman PM, Grant SF. Mendelian randomization in the era of genomewide association studies. Clin Chem. 2010;56(5):723–8. https://doi.org/10.1373/clinchem.2009.141564.
78. Tyrrell J, Richmond RC, Palmer TM, Feenstra B, Rangarajan J, Metrustry S, et al. Genetic evidence for causal relationships between maternal obesity-related traits and birth weight. JAMA. 2016;315(11):1129–40. https://doi.org/10.1001/jama.2016.1975.
79. Lee HA, Park EA, Cho SJ, Kim HS, Kim YJ, Lee H, et al. Mendelian randomization analysis of the effect of maternal homocysteine during pregnancy, as represented by maternal MTHFR C677T genotype, on birth weight. J Epidemiol. 2013;23(5):371–5.
80. Yajnik CS, Chandak GR, Joglekar C, Katre P, Bhat DS, Singh SN, et al. Maternal homocysteine in pregnancy and offspring birthweight: epidemiological associations and Mendelian randomization analysis. Int J Epidemiol. 2014;43(5):1487–97. https://doi.org/10.1093/ije/dyu132.
81. Bouthoorn SH, van Lenthe FJ, Kiefte-de Jong JC, Taal HR, Wijtzes AI, Hofman A, et al. Genetic taste blindness to bitter and body composition in childhood: a Mendelian randomization design. Int J Obes. 2014;38(7):1005–10. https://doi.org/10.1038/ijo.2013.141.
82. Zhang G, Bacelis J, Lengyel C, Teramo K, Hallman M, Helgeland Ø, et al. Assessing the causal relationship of maternal height on birth size and gestational age at birth: a Mendelian randomization analysis. PLoS Med. 2015;12(8):e1001865. https://doi.org/10.1371/journal.pmed.1001865.
83. Wang T, Huang T, Li Y, Zheng Y, Manson JE, Hu FB, Qi L. Low birthweight and risk of type 2 diabetes: a Mendelian randomisation study. Diabetologia. 2016;59(9):1920–7. https://doi.org/10.1007/s00125-016-4019-z.
84. Au Yeung SL, Lin SL, Li AM, Schooling CM. Birth weight and risk of ischemic heart disease: a Mendelian randomization study. Sci Rep. 2016;6:38420. https://doi.org/10.1038/srep38420.
85. Bernard JY, Pan H, Aris IM, Moreno-Betancur M, Soh SE, Yap F, et al. Long-chain polyunsaturated fatty acids, gestation duration, and birth size: a Mendelian randomization study using fatty acid desaturase variants. Am J Clin Nutr. 2018;108(1):92–100. https://doi.org/10.1093/ajcn/nqy079.

86. Geng TT, Huang T. Maternal central obesity and birth size: a Mendelian randomization analysis. Lipids Health Dis. 2018;17(1):181. https://doi.org/10.1186/s12944-018-0831-4.
87. Smith JA, Ware EB, Middha P, Beacher L, Kardia SL. Current applications of genetic risk scores to cardiovascular outcomes and subclinical phenotypes. Curr Epidemiol Rep. 2015;2(3):180–90.
88. Claussnitzer M, Dankel SN, Klocke B, Grallert H, Glunk V, Berulava T, et al. Leveraging cross-species transcription factor binding site patterns: from diabetes risk loci to disease mechanisms. Cell. 2014;156(1-2):343–58. https://doi.org/10.1016/j.cell.2013.10.058.
89. Miller GW, Jones DP. The nature of nurture: refining the definition of the exposome. Toxicol Sci. 2014;137(1):1–2. https://doi.org/10.1093/toxsci/kft251.
90. Wild CP. Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology. Cancer Epidemiol Biomarkers Prev. 2005;14(8):1847–50. https://doi.org/10.1158/1055-9965.EPI-05-0456.
91. Wild CP. The exposome: from concept to utility. Int J Epidemiol. 2012;41(1):24–32. https://doi.org/10.1093/ije/dyr236.
92. DeBord DG, Carreón T, Lentz TJ, Middendorf PJ, Hoover MD, Schulte PA. Use of the "exposome" in the practice of epidemiology: a primer on -omic technologies. Am J Epidemiol. 2016;184(4):302–14. https://doi.org/10.1093/aje/kwv325.
93. Vineis P, Chadeau-Hyam M, Gmuender H, Gulliver J, Herceg Z, Kleinjans J, et al. The exposome in practice: design of the EXPOsOMICS project. Int J Hyg Environ Health. 2017;220(2 Pt A):142–51. https://doi.org/10.1016/j.ijheh.2016.08.001.
94. Turner MC, Vineis P, Seleiro E, Dijmarescu M, Balshaw D, Bertollini R, et al. EXPOsOMICS: final policy workshop and stakeholder consultation. BMC Public Health. 2018;18(1):260. https://doi.org/10.1186/s12889-018-5160-z.
95. Hanson MA, Gluckman PD. Early developmental conditioning of later health and disease: physiology or pathophysiology? Physiol Rev. 2014;94(4):1027–76. https://doi.org/10.1152/physrev.00029.2013.
96. Chatterjee N, Shi J, García-Closas M. Developing and evaluating polygenic risk prediction models for stratified disease prevention. Nat Rev Genet. 2016;17(7):392–406. https://doi.org/10.1038/nrg.2016.27.
97. Imura H. Life course health care and preemptive approach to non-communicable diseases. Proc Jpn Acad Ser B Phys Biol Sci. 2013;89(10):462–73. https://doi.org/10.2183/pjab.89.462.
98. Sata F. Developmental origins of health and disease (DOHaD) cohorts and interventions: status and perspective. In: Sata F, Fukuoka H, Hanson M, editors. Pre-emptive medicine: public health aspects of developmental origins of health and disease. Singapore: Springer; 2019. p. 53–70.
99. https://www.amed.go.jp/en/program/list/04/02/002.html. Accessed 23 May 2019.

Chapter 20
Epigenetics: Strategies for Prevention Research

Wilfried Karmaus, Ali H. Ziyab, and Nandini Mukherjee

Abstract Being etiologically positioned between the genome and the environment, epigenetic markers (e.g., DNA methylation) represent the cumulative memory of genetic susceptibility and environmental exposures. Hence, the premise of epigenetics is that it can serve as a biomarker of the interplay between genetic predisposition and current and past environmental exposures. Such biomarkers can identify at-risk subgroups and related past and current exposures, supportive in devising mitigating and preventive strategies. As explained in this chapter, exposures to environmental chemicals result in epigenetic modifications that, conditional on genetic susceptibilities, mediate exposure effects on disease development. However, to gain better insights on the link between environmental exposures, epigenetic modifications, and disease mechanisms, researchers need to develop and incorporate epidemiologic methods and strategies that can explain how epigenetics might mediate the influence of environmental exposures on disease development. Once causal and molecular pathways are elucidated, epigenetic markers will serve as diagnostic and therapeutic targets facilitating the vision of personalized precision medicine.

Keywords Epigenetics · DNA methylation · Environmental exposures Chemicals · Disease markers · Prevention

W. Karmaus (✉) · N. Mukherjee
University of Memphis, Memphis, TN, USA
e-mail: karmaus1@memphis.edu; nmkhrjee@memphis.edu

A. H. Ziyab
Faculty of Medicine, Kuwait University, Kuwait City, Kuwait
e-mail: aziyab@hsc.edu.kw

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_20

20.1 Epigenetic Markers

In the 1940s, Conrad Waddington used the term epigenetics to describe how the genotype manifests itself as a phenotype [1]. In 1958, David Nanney borrowed the term to describe inherited phenomena that could not be explained by conventional genetics [2]. In 2007, epigenetics has been defined by Mark Ptashne using three criteria: (1) a change in the activity of a gene that does not involve a mutation, (2) that is initiated by a signal, and (3) that is inherited (mitotically or meiotically) in the absence of the signal that initiated the change [3]. Four epigenetic markers have been identified: (a) DNA methylation, (b) histone modification, (c) remodeled chromatin, and (d) small (21- to 26-nt) and non-coding RNAs. There is evidence that DNA methylation (DNAm) fulfills all three of the above criteria [4–6]. Histone modifications satisfy some of the criteria for being an epigenetic mechanism in that they can result from exogenous signals such as cigarette smoke and alter gene activity [7–9]. However, meiotic inheritance of histone modifications has only been demonstrated in *C. elegans* [10]. DNAm usually works hand in hand with histone modifications to activate or silence genes by influencing chromatin structure and its accessibility by transcription factors [11]. Hence, it is possible that DNAm constitutes a mechanism of mitotic inheritance for some histone modifications. Given the complex and ever-changing structure of chromatin, there is little information on chromatin remodeling regarding initiation, alteration of gene activity, and inheritance [12–14].

MicroRNAs (miRNAs) also have been shown to be controlled by exogenous factors and to alter gene activity by either inhibiting translation or degrading messenger RNAs (mRNA) [15, 16]. For instance, in humans, miRNAs have been demonstrated to be differentially expressed in current and never smokers and to be related to particulate matter exposure [7, 17]. Currently, there is little evidence that environmentally induced miRNAs expression patterns can be inherited [18]. However, non-coding RNA is considered to transmit epigenetic information from one generation to the next [19–22]. It is reported that DNAm also plays an essential role in the regulation on long non-coding RNA [23]. Thus, it is possible that DNAm affects the activity of miRNAs and facilitates their inheritance [23].

Hence, in the following chapter, we will focus on the truly and well-established epigenetic mechanism of DNAm, which is characterized by the addition of a methyl or hydroxymethyl group to the carbon 5′ position of cytosine, occurring in the sequence of CG dinucleotides. These are called cytosine-phosphate-guanine dinucleotides or CpGs. Recently, non-CpG methylation in a CHH and CHG context (where H = A [adenine], C [cytosine], or T [thymine]), existing in embryonic stem cells, were detected [24]. However, these characteristics have not been further investigated. Hence this chapter focuses on DNAm of the CpG sites.

20.2 Explanatory Epigenetic Models

Exposure to environmental chemicals results from many different nutritional, behavioral, chemical, and physical influences [25–28]. Mechanisms of how environmental chemicals (EC) alter DNAm remain unknown [27]. DNAm is considered to be regulated by DNA methyl-transferases (DNMTs) that catalyze the transfer of a methyl-group from S-adenosyl methionine to the carbon 5′ position of the cytosine [29, 30]. Demethylation seems to result due to ten-eleven translocation family (TET) proteins that oxidize the methyl-group to hydroxymethyl, formyl, or carboxyl groups [31, 32]. However, it remains unclear why specific DNA sequences or genes are affected by specific ECs resulting in changes of DNAm and others are not. Various similar explanatory models have been proposed to describe the process of how effects of ECs can be mediated via DNAm on the disease, changing its risk (Fig. 20.1) [33, 34].

DNAm is also considered to be influenced by genetic polymorphisms, also called methylation quantitative trait loci (methQTL) [35]. Additionally, environmental stressor, together with the respective polymorphisms, may result in alterations of DNAm [33]. In the first step, there seems to be an interaction of chemical exposures with a specific methQTL altering the methylations of a specific CpG on a disease-related gene (Fig. 20.1). In the second step, the methylation of a specific CpG on a specific gene can modify the disease potential of this gene (modifiable genetic variant = modGV). For instance, the methylation can block the activity of the gene promoter [34, 36] or a CpG in the gene body may modify splicing resulting in the production of different messenger RNAs [37]. By allowing the generation of multiple messenger RNA products from a single gene, alternative splicing increases the diversity of transcripts and proteins bringing about a change in the phenotype, ultimately changing the disease risk. Considering that more than 90% of human genes undergo splicing [38], it is likely that changes in DNAm may potentially influence the end product of most human genes.

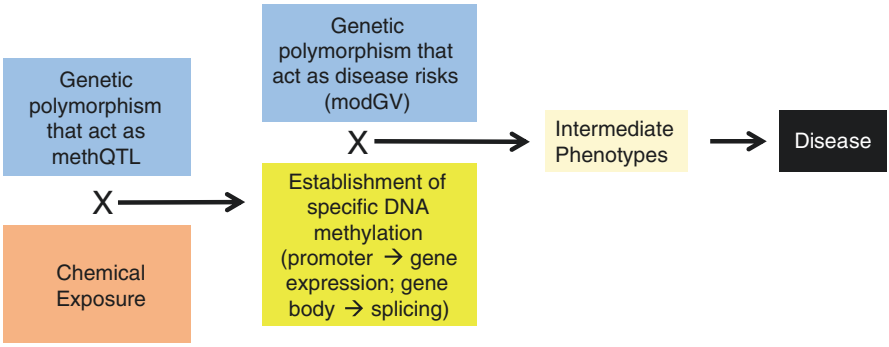


Fig. 20.1 Two-stage interaction model of chemical exposure, genetic variants, and DNA methylation

20.3 Time Order

When considering epigenetic models explaining a change of disease risks, we need to reflect on the time order. In epigenetic studies, we assume that epigenetic markers constitute the link between exposure and disease: exposure → epigenetic → disease (Fig. 20.2). An optimal approach is to measure exposures in individuals not affected by the disease of interest, then, in an adjacent time window, to determine epigenetic markers, and finally to follow participants and determine their disease status. Nevertheless, there are still multiple possibilities for reverse associations ("causality"). First, epigenetic markers may affect metabolism (such as of lipids) leading to a differential measurement of the body burden of exposure (such as lipophilic exposures). Second, processes affecting the underlying mechanisms of the disease of interest present before the disease may affect epigenetic markers, making it difficult to eliminate possibilities of reverse "causation."

Effect of reverse associations can be reduced if we start with healthy newborns, measure epigenetic markers shortly after birth (in DNA from cord blood or from dried blood samples collected as part of newborn screening), and assess their disease status later in childhood. This framework concurs with developmental origins of health and disease (DOHaD) hypothesis (also called the Barker hypothesis) [39], which suggests that early life conditions influence the susceptibility for diseases later in life [40]. In this setting, DNAm is considered to be the plausible mediator of in utero exposure to offspring disease [41] satisfying an appropriate forward time order. In some studies, epigenetic markers, predictive of asthma and BMI that developed later in childhood [42, 43], have been measured in cord blood, successfully avoiding reverse causations (disease → epigenetics).

Other examples include CpGs from cord and heel prick blood, respectively, that were associated with phenylketonuria [44], neurodevelopmental disorders [45], and childhood adiposity [43]. Wang et al. (2013) found prenatal maternal smoking to be associated with 5′-CpG island (CGI) thymic stromal lymphopoietin (*TSLP*) in cord blood, which in turn was found to be related to eczema at

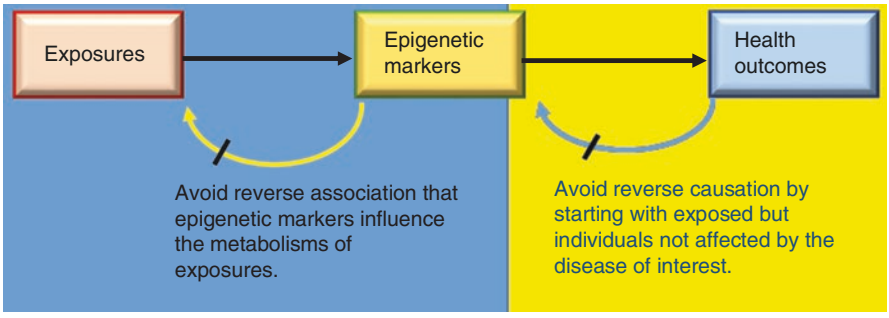


Fig. 20.2 Time order of exposures, epigenetic markers, and health outcomes

2 years of age [46]. Perera et al. (2009) reported 5′-CpG Island of *ACSL3* linked to transplacental exposure to airborne polycyclic aromatic hydrocarbons (PAHs) and childhood asthma symptoms prior to age 5 years, [47], while Cardenas et al. (2017) identified a persistent epigenetic change of the Paraoxonase 1 gene (*PON1*), shown to be related to prenatal mercury exposure and cognitive performance during childhood [48].

20.4 Tissue Specificity

Although the above findings suggest a role of DNAm measured in blood samples linking in utero exposures to diseases later in life, there are still potential caveats to overcome. Evaluating disease association using whole (cord) blood methylation has inherently been under debate, since it may not appropriately reflecting the epigenetic profile of target tissues [41]. Considering in utero origins of disease, one can consider, on the one hand, that DNAm reprogramming due to environmental exposure may affect cells of all cell layers (ecto-, meso-, and endoderm) similarly or alternatively cells of the ecto-, meso-, and endoderm differently, however, resulting in a stable epigenome of the post-mitotic cells. In such a setting, either all tissue of all cell layers may share DNAm characteristics or tissue of a specific cell-layer shares epigenome-wide DNAm characteristics (e.g., mesoderm: blood, lung, etc.). On the other hand, when exposure-events and DNAm reprogramming occur later in development it may result in a different repertoire of DNAm in different tissues and cell-subtypes [49]. Characterizing the DNAm of multiple tissues (blood, buccal, bronchial, or skin cells) in the Roadmap Epigenomics project [50] provides some information on similar and dissimilar DNAm in different tissue. However, in addition, it is necessary to consider the timing of the intra-uterine conditions when the DNAm was reprogrammed (before, during, or after the development of ecto-, meso-, and endoderm) if we want to compare methylation characteristics of specific genes in different tissues.

20.5 Small DNA Methylation Differences: Large Effects?

There are differing opinions regarding acceptable effect sizes of exposure-methylation and methylation-disease associations [37]. Often small differences in the methylation of a CpG, for instance, a 2% difference in white blood cells may impact gene expression and influence several downstream molecular mechanisms [47] leading to large effects. To understand this, we have to consider that a CpG of one specific cell-type such as natural killer cells (about 2% of white blood cells) may be 100% differentially methylated, resulting in a disease. However, this difference is detected only in about 2% of the white blood cells. Hence, overall small differential DNAm with large effects are reasonable.

20.6 Environment and DNA Methylation

Positioned "between" the genome and the environment, DNAm represents the cumulative memory of genetic susceptibility and environmental exposures (signatures, or finger- or footprint). The reprogrammed DNAm may be mitotically inherited and has been shown, e.g., in the case of prenatal smoking, to persist over a long period of time (>50 years) [51]. Hence, DNAm can serve as a biomarker of the interplay between genetic predisposition and environmental exposures. Following the idea of a clear time order, we focus again on the role of exposure to environmental chemicals during early developmental stages that may result in differential DNAm and diseases. In the following section we provide examples of four different chemical exposures: tobacco smoking, traffic-related air pollution, persistent organic pollutant, and arsenic.

Tobacco smoking and addiction remains a global public health challenge that is associated with harmful health-related consequences. Around 5000 toxic and carcinogenic chemical mixtures are found in cigarette smoke, of which, 98 compounds have been classified as hazardous smoke components [52]. Maternal smoking during pregnancy has been linked to several adverse health-related outcomes in the offspring over the life-span; for instance, low birth weight [53, 54], reduced lung function [55, 56], childhood and adulthood obesity [57–59], and neurodevelopmental disorders [60, 61]. Hence, it is essential to have sensitive biomarker(s) to identify and categorize individuals with current and past exposure to smoking (see below Fig. 20.3). Thus far, cotinine, a metabolite of nicotine, has been used as a biomarker of cigarette smoking. However, cotinine has a short half-life of 16–19 h [62], which limits its use only as a short-term biomarker. However, the search for a novel biomarker of gestational smoking exposure has succeeded. Joubert and colleagues, examined alterations in DNAm at an epigenome-wide scale, identified changes in DNAm measured in cord blood of newborns that were strongly and consistently

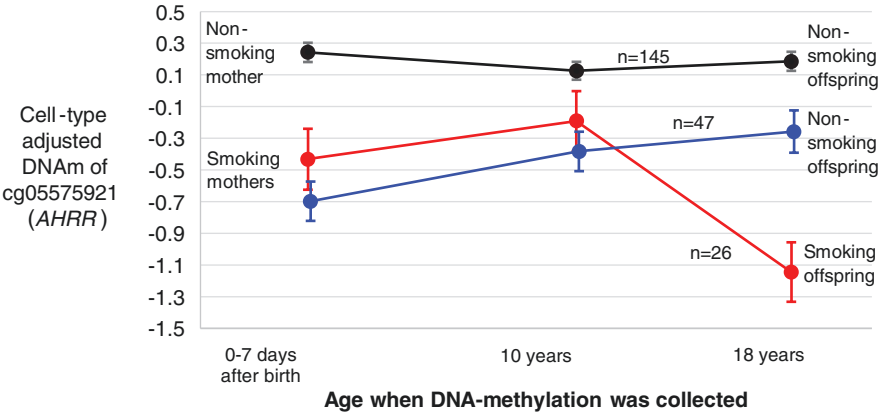


Fig. 20.3 Trajectory of cg05575921 (*AHRR* gene) from birth to age 18 years

associated with exposure to gestational smoking [63]. Differential DNAm were identified and replicated in the *AHRR* and *CYP1A1* (xenobiotic-detoxifying genes) and *GFI1* genes. Subsequently, several research groups have confirmed the previously noted associations and added more smoking-induced DNAm signatures [64–66]. More recently, addressing the methylation fingerprint of maternal smoking across the epigenome, the Pregnancy And Childhood Epigenetics (PACE) consortium reported results from 13 birth cohort studies [28]. Results of this meta-analysis confirmed the previously reported associations and further added novel differentially methylated CpGs related to smoking. Therefore, accumulated evidence suggests that DNAm of specific genomic loci can serve as long-term biomarkers of exposure to smoking [51] and may also act as predispositions for diseases.

Similar to cigarette smoke exposure, traffic-related air pollution (TRAP) resembles exposure to a multitude of toxic gaseous pollutants (mainly nitrogen oxides) and particulate components that have been associated with numerous adverse health outcomes [67–72]. Of particular concern are diesel exhaust particles (DEPs), accounting for up to 90% of particulate matter (PM) related to traffic emissions. DEPs have been linked to acute and severe health consequences [73, 74]. Novel findings also suggest that exposures to TRAP are associated with differential DNAm [73, 75–77]. Multiple studies have shown associations between exposure to PM and DNAm levels at the inducible nitric oxide synthase gene (*iNOS*) [73, 76, 78, 79]. Also, exposures to TRAP during the first year of life were associated with higher methylation of CpGs on the *FOX3* gene, which subsequently were linked with persistent wheezing and asthma at 7 years of age [80]. Similarly, promoter methylation of *TET1* gene was found to be associated with TRAP exposure [81]. In line with the early life origins of health and disease hypothesis, maternal exposure to traffic-related polycyclic aromatic hydrocarbons (PAHs) during pregnancy has been shown to be associated with differential cord blood DNAm in *ACSL3* gene [47]. Moreover, increased methylation of *IFN-γ* gene measured in cord blood was related to gestational exposure to traffic-related PAH [82]. A constraint is that the aforementioned studies used a candidate-gene approach when assessing associations between TRAP exposure and DNAm, possibly ignoring other unknown effects. However, recently, Gruzieva and colleagues (2017) conducted an epigenome-wide meta-analysis to evaluate the role of prenatal exposure to nitrogen dioxide (NO_2), as an indicator of TRAP, with cord blood DNA methylation levels across the genome in newborns [83]. Results of this large meta-analysis showed that maternal exposure to NO_2 during gestation was associated with differential DNAm in offspring cord blood particularly of genes involved in mitochondria function and antioxidant defense pathways. Thus, DNAm could also serve as biomarker for in utero exposure to TRAP; however, specific markers need to be identified.

Exposure, specifically in utero, to persistent organic pollutants (POPs) and endocrine disrupters (EDs) have been linked to developmental anomalies and adverse health effects [84–86]. For instance, maternal serum concentrations of POPs during pregnancy were associated with offspring risk of having asthma 20 years later [87]. The long-term effect may be related to the persistency of the body burden of POPs, their life-long bioaccumulation or through DNAm mediation. For example, maternal

serum concentrations of perfluorooctanoate (PFOA) were associated with lower DNAm in cord blood [88]. Similarly, POPs measured in maternal serum during late pregnancy showed association with DNAm levels at birth [89]. Specifically, elevated maternal blood polybrominated diphenyl ethers (PBDE) levels were associated with lower cord blood *TNF-α* promoter methylation [90]. These findings suggest that DNAm may act as mediator between POP exposures and the onset of diseases. Nevertheless, there is a need to identify specific mediators.

Arsenic, specifically in its inorganic form, is toxic and is commonly present in contaminated groundwater around the world. The greatest arsenic-related public health risk arises by using the contaminated water for drinking, preparing food, and irrigation of food crops. Although exposure to arsenic has been linked to adverse pregnancy outcomes [91], the mechanisms underlying arsenic susceptibility to disease development are not fully understood. Epigenetic alterations have been speculated to mediate arsenic-related adverse health effects [92]. Associations, concurrent and in utero, between arsenic exposure and DNAm have been reported, reviewed in Pacchierotti (2015) [92]. Of interest, multiple epigenome-wide investigations have revealed associations between maternal arsenic exposure and offspring cord blood DNAm [93–96]. Collectively, these studies show differential DNAm of genes implicated in birth outcomes (gestational age and head circumference), cancer, cardiovascular diseases, diabetes mellitus, and low-density lipoprotein cholesterol.

The current state of knowledge suggests that exposure to various environmental chemicals during critical developmental stages, e.g., in utero, modulate the DNAm, which in turn could act as risk factors for the development of health-related outcomes. Hence, if replicated, multiple environmental-chemical induced specific CpGs can be used in the future as biomarkers of past as well as current exposures. Such biomarkers will help to identify individuals early in life who might benefit from intervention to mitigate the effects of past and current exposures.

20.7 DNA Methylation and Subsequent Diseases

DNA methylation is considered to effectively mediate in utero exposures since it results in exposure-specific methylation levels in response to diet [97, 98], alcohol intake [99, 100], stress [43, 101, 102], and smoking during pregnancy [28, 103, 104]. In particular, the CpG site cg05575921 (*AHRR* gene) has been established as a biomarker of the tobacco smoking [65]. In support of the pathogenetic relevance of differential DNAm, it has been demonstrated that differential methylation is associated with altered gene expression [46, 105, 106].

In addition to its association with gestational exposures, candidate-gene and epigenome-wide approaches have established associations of DNAm of specific epigenetic loci with congenital diseases [107, 108], cancer [109–112], obesity [113, 114], and asthma and allergic diseases [115–118]. Although there are challenges to comparably define both exposures and health outcomes, meta-analyses have successfully associated multiple CpG sites with diseases across various populations

[119–121]. However, many of these studies have measured DNAm and disease outcomes concurrently; hence, the possibility of a reverse causation cannot be ruled out. In addition, specific postnatal DNAm may also involve additional exposures [122, 123] and may result from gene-environment interactions [33, 124]. Hence, there are challenges untangling confounding effects and effects of other exposures. In addition, to understand various pathways from exposure via DNAm to diseases, it is necessary either to use CpG sites that have been determined as fingerprints or signatures of exposures or to apply statistical approaches of structural equation analyses to test for mediation [125–127] from exposure via differential epigenetics to diseases, involving a clear time order.

20.8 Future Strategies to Improve Etiologic Research Using Epigenetics

We believe that the knowledge gained from understanding of the role of epigenetics, particularly DNAm, and of links between exposures and diseases, is highly significant. In the future, we may learn on how to mitigate adverse pathogenetic processes that are related to DNAm. Regarding past exposures, the detection and validation of epigenetic markers reflecting the memory of past environmental exposures are also instrumental for future research. With regard to diseases, epigenetic risk factors may provide novel insights into many pathways that have not been detected in genetic research (missing heritability) [128, 129]. However, to link exposure and disease by mediating DNAm, it is not only necessary to have signatures (fingerprints) of exposures but also to have these fingerprints linked to diseases.

The research challenge of assessing epigenetic effects is overwhelming and no single study, even focused on specific exposures, a single DNAm site, and a single disease, is able to establish such associations without replications. Thus, we need to develop improved research structures to overcome isolated work and support collaborations and cross-study comparisons. One example is the Pregnancy And Childhood Epigenetics (PACE) group at the National Institute of Environmental Health Sciences. This group includes researchers around the world in the field of epigenetics who are interested in studying the early life environmental impacts on human disease [130]. Collaborative structures have also been developed in Asia [131, 132] and Europe (enrieco [133] or EPIGENESYS [134], just to mention a few); however, the latter seems to miss a long-term survival.

Regarding education and training in public health sciences, particularly in epidemiology, there is a lack of methodological training in studies that can investigate pathways form exposures via epigenetics to disease. Currently the main focus is on approaches that simplify associations using directed acyclic graphs, ignoring the complexity and plurality of mediating processes [135]. However, to understand multiple mediation processes of differently methylation gene-sites that may link exposures and disease, we need to employ more complex pathway analyses in research and training of new investigators [136, 137].

Beyond having unambiguous directions from exposure via epigenetics to disease, we also need to consider how specific epigenetic markers develop over time. DNAm as biomarker can retain the memory even after 50 years as shown for prenatal smoke exposure by research from Richmond et al. [51]. However, other CpG sites show specific patterns over time. For instance, cg07555921 (not detected as stable CpG in the work by Richmond et al.) was measured at birth, at age 10, and age 18 years in the Isle of Wight birth cohort [138]. This CpG site shows, determined via group-based trajectory modeling [139], three separated patterns. When the mother did not smoke, the methylation level adjusted for blood cells at the different ages was higher and remained high when the offspring did not smoke tobacco at age 18 years (Fig. 20.3).

If the mother smoked, the methylation level was lower, but recovered in children who did not initiate smoking. However, if the offspring started smoking, then the methylation of cg07555921 experience another reduction in methylation, showing three separated groups at age 18 years (Fig. 20.3). These findings suggest, first, that some CpGs can be used as biomarkers of intervention or as therapeutic biomarkers [140], indicating whether an intervention or a therapy was effective, even if the final disease has not been established yet. The development of CpGs over age can also show us whether methylation is stable, recovers, or is further altered *when the exposure is repeated. Second, those patterns can help to distinguish whether a single exposure during pregnancy or repeated exposures are more likely to result in diseases in adulthood.*

Finally, given that specific CpG sites act as intervening variables or mediators of adverse exposure effects on health, we need to find out whether there are opportunities to change methylation levels of specific sites (Fig. 20.3), targeting sites that also act as disease risks. If we can change the DNAm at these target sites, then there is a chance to reduce disease risks related to exposures. It is particularly important to identify such target CpGs in newborns associated with exposures that occurred in utero, since, in such cases, the exposure status cannot be changed or prevented any longer once a child was exposed. However, we can prevent or alter the conditions under which an exposure establishes its detrimental effects by intervening on the levels of CpG even later in life. For instance, we have shown that tetanus vaccination is associated with differential methylation of two CpG sites; one located within the *KIAA1549L* gene and the other is located in an intergenic region between *PSMG3* and *TFAMP1* genes [115]. The differential methylation of these two CpG sites was related to a lower risk of asthma, suggesting that there exist untapped opportunities to prevent diseases by changing DNAm. These novel prospects, whose potential has not yet received sufficient attention, also distinguish genetic from the epigenetic risk factors.

20.9 Conclusions

Exposure to environmental chemicals during vulnerable developmental periods has demonstrated detrimental health effects. These adverse effects are often mediated by changes involving epigenetic mechanisms. First, we need to establish more and

sustained collaboration to identify and replicate some thousand important epigenetic markers in a shorter time-frame. Second, we need to learn to apply structural equation models to adequately address the mediating effects of epigenetic markers. Third, we need to understand how epigenetic markers develop over time and whether different developments involve different disease risks. Fourth, we need to identify those epigenetic markers that act as mediators between exposure and diseases. Such epigenetic biomarkers can then be employed to prevent or mitigate exposure-initiated diseases. Finally, we need to gain a better understanding of the molecular pathways of epigenetic markers and test whether they can serve as diagnostic and therapeutic targets to realize the vision of personalized precision medicine.

References

1. Ledford H. Language: disputed definitions. Nature. 2008;455:1023–8.
2. Nanney DL. Epigenetic control systems. Proc Natl Acad Sci U S A. 1958;44:712–7.
3. Ptashne M. On the use of the word 'epigenetic'. Curr Biol. 2007;17:R233–R6.
4. Daxinger L, Whitelaw E. Understanding transgenerational epigenetic inheritance via the gametes in mammals. Nat Rev Genet. 2012;13:153–62.
5. Alegria-Torres JA, Baccarelli A, Bollati V. Epigenetics and lifestyle. Epigenomics. 2011;3:267–77.
6. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012;13:484–92.
7. Lovinsky-Desir S, Miller RL. Epigenetics, asthma, and allergic diseases: a review of the latest advancements. Curr Allergy Asthma Rep. 2012;12:211–20.
8. Clifford RL, John AE, Brightling CE, Knox AJ. Abnormal histone methylation is responsible for increased vascular endothelial growth factor 165a secretion from airway smooth muscle cells in asthma. J Immunol. 2012;189:819–31.
9. Royce SG, Karagiannis TC. Histone deacetylases and their role in asthma. J Asthma. 2012;49:121–8.
10. Greer EL, Maures TJ, Ucar D, Hauswirth AG, Mancini E, Lim JP, et al. Transgenerational epigenetic inheritance of longevity in Caenorhabditis elegans. Nature. 2011;479:365–71.
11. Callinan PA, Feinberg AP. The emerging science of epigenomics. Hum Mol Genet. 2006;15(suppl_1):R95–101.
12. Travers AA, Vaillant C, Arneodo A, Muskhelishvili G. DNA structure, nucleosome placement and chromatin remodelling: a perspective. Biochem Soc Trans. 2012;40:335–40.
13. Berr A, Menard R, Heitz T, Shen WH. Chromatin modification and remodelling: a regulatory landscape for the control of Arabidopsis defence responses upon pathogen attack. Cell Microbiol. 2012;14:829–39.
14. Grigoryev SA, Woodcock CL. Chromatin organization - the 30 nm fiber. Exp Cell Res. 2012;318:1448–55.
15. Angulo M, Lecuona E, Sznajder JI. Role of MicroRNAs in lung disease. Arch Bronconeumol. 2012;48:325–30.
16. Su WY, Xiong H, Fang JY. Natural antisense transcripts regulate gene expression in an epigenetic manner. Biochem Biophys Res Commun. 2010;396:177–81.
17. Yang IV, Schwartz DA. Epigenetic control of gene expression in the lung. Am J Respir Crit Care Med. 2011;183:1295–301.
18. Buckley BA, Burkhart KB, Gu SG, Spracklin G, Kershner A, Fritz H, et al. A nuclear Argonaute promotes multigenerational epigenetic inheritance and germline immortality. Nature. 2012;489:447.

19. Heard E, Martienssen RA. Transgenerational epigenetic inheritance: myths and mechanisms. Cell. 2014;157:95–109.
20. Sales VM, Ferguson-Smith AC, Patti ME. Epigenetic mechanisms of transmission of metabolic disease across generations. Cell Metab. 2017;25:559–71.
21. Wu H, Hauser R, Krawetz SA, Pilsner JR. Environmental susceptibility of the sperm epigenome during windows of male germ cell development. Curr Environ Health Rep. 2015;2:356–66.
22. Sheng L, Ye L, Zhang D, Cawthorn WP, Xu B. New insights into the long non-coding RNA SRA: physiological functions and mechanisms of action. Front Med. 2018;5:244.
23. Wu W, Bhagat TD, Yang X, Song JH, Cheng Y, Agarwal R, et al. Hypomethylation of noncoding DNA regions and overexpression of the long noncoding RNA, AFAP1-AS1, in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterology. 2013;144:956–66.e4.
24. Kurdyukov S, Bullock M. DNA methylation analysis: choosing the right method. Biology. 2016;5:E3.
25. Berti C, Agostoni C, Davanzo R, Hypponen E, Isolauri E, Meltzer HM, et al. Early-life nutritional exposures and lifelong health: immediate and long-lasting impacts of probiotics, vitamin D, and breastfeeding. Nutr Rev. 2017;75:83–97.
26. Jirtle RL, Skinner MK. Environmental epigenomics and disease susceptibility. Nat Rev Genet. 2007;8:253–62.
27. Torano EG, Garcia MG, Fernandez-Morera JL, Nino-Garcia P, Fernandez AF. The impact of external factors on the epigenome: in utero and over lifetime. Biomed Res Int. 2016;2016:2568635.
28. Joubert BR, Felix JF, Yousefi P, Bakulski KM, Just AC, Breton C, et al. DNA methylation in newborns and maternal smoking in pregnancy: genome-wide consortium meta-analysis. Am J Hum Genet. 2016;98:680–96.
29. Jurkowska RZ, Jeltsch A. Enzymology of mammalian DNA methyltransferases. Adv Exp Med Biol. 2016;945:87–122.
30. Castillo-Aguilera O, Depreux P, Halby L, Arimondo PB, Goossens L. DNA methylation targeting: the DNMT/HMT crosstalk challenge. Biomol Ther. 2017;7:3.
31. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324:930–5.
32. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science. 2011;333:1300–3.
33. Karmaus W, Ziyab AH, Everson T, Holloway JW. Epigenetic mechanisms and models in the origins of asthma. Curr Opin Allergy Clin Immunol. 2013;13:63–9.
34. Feinberg AP. The key role of epigenetics in human disease prevention and mitigation. N Engl J Med. 2018;378:1323–34.
35. Bell JT, Pai AA, Pickrell JK, Gaffney DJ, Pique-Regi R, Degner JF, et al. DNA methylation patterns associate with genetic and gene expression variation in HapMap cell lines. Genome Biol. 2011;12:R10.
36. Baylin SB, Ohm JE. Epigenetic gene silencing in cancer—a mechanism for early oncogenic pathway addiction? Nat Rev Cancer. 2006;6:107–16.
37. Lev Maor G, Yearim A, Ast G. The alternative role of DNA methylation in splicing regulation. Trends Genet. 2015;31:274–80.
38. Laurent L, Wong E, Li G, Huynh T, Tsirigos A, Ong CT, et al. Dynamic changes in the human methylome during differentiation. Genome Res. 2010;20:320–31.
39. Dover GJ. The Barker hypothesis: how pediatricans will diagnose and prevent common adult-onset diseases. Trans Am Clin Climatol Assoc. 2009;120:199–207.
40. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61–73.
41. Saffery R, Novakovic B. Epigenetics as the mediator of fetal programming of adult onset disease: what is the evidence? Acta Obstet Gynecol Scand. 2014;93:1090–8.
42. DeVries A, Vercelli D. The neonatal methylome as a gatekeeper in the trajectory to childhood asthma. Epigenomics. 2017;9:585–93.

43. Wu S, Gennings C, Wright RJ, Wilson A, Burris HH, Just AC, et al. Prenatal stress, methylation in inflammation-related genes, and adiposity measures in early childhood: the PROGRESS cohort study. Psychosom Med. 2018;80:34.
44. Beyan H, Down TA, Ramagopalan SV, Uvebrant K, Nilsson A, Holland ML, et al. Guthrie card methylomics identifies temporally stable epialleles that are present at birth in humans. Genome Res. 2012;22:2138–45.
45. Lillycrop KA, Costello PM, Teh AL, Murray RJ, Clarke-Harris R, Barton SJ, et al. Association between perinatal methylation of the neuronal differentiation regulator HES1 and later childhood neurocognitive function and behaviour. Int J Epidemiol. 2015;44:1263–76.
46. Wang IJ, Chen SL, Lu TP, Chuang EY, Chen PC. Prenatal smoke exposure, DNA methylation, and childhood atopic dermatitis. Clin Exp Allergy. 2013;43:535–43.
47. Perera F, Tang WY, Herbstman J, Tang D, Levin L, Miller R, et al. Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. PLoS One. 2009;4:e4488.
48. Cardenas A, Rifas-Shiman SL, Agha G, Hivert MF, Litonjua AA, DeMeo DL, et al. Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. Sci Rep. 2017;7:288.
49. Lappalainen T, Greally JM. Associating cellular epigenetic models with human phenotypes. Nat Rev Genet. 2017;18:441–51.
50. Roadmap Epigenomics Consortium, Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015;518:317–30.
51. Richmond RC, Suderman M, Langdon R, Relton CL, Davey Smith G. DNA methylation as a marker for prenatal smoke exposure in adults. Int J Epidemiol. 2018;47:1120–30.
52. Talhout R, Schulz T, Florek E, van Benthem J, Wester P, Opperhuizen A. Hazardous compounds in tobacco smoke. Int J Environ Res Public Health. 2011;8:613–28.
53. Pereira PP, Da Mata FA, Figueiredo AC, de Andrade KR, Pereira MG. Maternal active smoking during pregnancy and low birth weight in the Americas: a systematic review and meta-analysis. Nicotine Tob Res. 2017;19:497–505.
54. Suter MA, Anders AM, Aagaard KM. Maternal smoking as a model for environmental epigenetic changes affecting birthweight and fetal programming. Mol Hum Reprod. 2013;19:1–6.
55. Balte P, Karmaus W, Roberts G, Kurukulaaratchy R, Mitchell F, Arshad H. Relationship between birth weight, maternal smoking during pregnancy and childhood and adolescent lung function: a path analysis. Respir Med. 2016;121:13–20.
56. McEvoy CT, Spindel ER. Pulmonary effects of maternal smoking on the fetus and child: effects on lung development, respiratory morbidities, and life long lung health. Paediatr Respir Rev. 2017;21:27–33.
57. Ziyab AH, Karmaus W, Kurukulaaratchy RJ, Zhang H, Arshad SH. Developmental trajectories of body mass index from infancy to 18 years of age: prenatal determinants and health consequences. J Epidemiol Community Health. 2014;68:934–41.
58. Rayfield S, Plugge E. Systematic review and meta-analysis of the association between maternal smoking in pregnancy and childhood overweight and obesity. J Epidemiol Community Health. 2017;71:162–73.
59. Behl M, Rao D, Aagaard K, Davidson TL, Levin ED, Slotkin TA, et al. Evaluation of the association between maternal smoking, childhood obesity, and metabolic disorders: a National Toxicology Program Workshop Review. Environ Health Perspect. 2013;121:170–80.
60. Clifford A, Lang L, Chen R. Effects of maternal cigarette smoking during pregnancy on cognitive parameters of children and young adults: a literature review. Neurotoxicol Teratol. 2012;34:560–70.
61. Chudal R, Brown AS, Gissler M, Suominen A, Sourander A. Is maternal smoking during pregnancy associated with bipolar disorder in offspring? J Affect Disord. 2015;171:132–6.
62. Jarvis MJ, Russell MA, Benowitz NL, Feyerabend C. Elimination of cotinine from body fluids: implications for noninvasive measurement of tobacco smoke exposure. Am J Public Health. 1988;78:696–8.

63. Joubert BR, Haberg SE, Nilsen RM, Wang X, Vollset SE, Murphy SK, et al. 450K epigenome-wide scan identifies differential DNA methylation in newborns related to maternal smoking during pregnancy. Environ Health Perspect. 2012;120:1425–31.
64. Lee KW, Pausova Z. Cigarette smoking and DNA methylation. Front Genet. 2013;4:132.
65. Shenker NS, Ueland PM, Polidoro S, van Veldhoven K, Ricceri F, Brown R, et al. DNA methylation as a long-term biomarker of exposure to tobacco smoke. Epidemiology. 2013;24:712–6.
66. Breton CV, Siegmund KD, Joubert BR, Wang X, Qui W, Carey V, et al. Prenatal tobacco smoke exposure is associated with childhood DNA CpG methylation. PLoS One. 2014;9:e99716.
67. Bourdrel T, Bind MA, Bejot Y, Morel O, Argacha JF. Cardiovascular effects of air pollution. Arch Cardiovasc Dis. 2017;110:634.
68. Carlsten C, Rider CF. Traffic-related air pollution and allergic disease: an update in the context of global urbanization. Curr Opin Allergy Clin Immunol. 2017;17:85–9.
69. Guarnieri M, Balmes JR. Outdoor air pollution and asthma. Lancet. 2014;383:1581–92.
70. Pedersen M, Stayner L, Slama R, Sorensen M, Figueras F, Nieuwenhuijsen MJ, et al. Ambient air pollution and pregnancy-induced hypertensive disorders: a systematic review and meta-analysis. Hypertension. 2014;64:494–500.
71. Carre J, Gatimel N, Moreau J, Parinaud J, Leandri R. Does air pollution play a role in infertility?: a systematic review. Environ Health. 2017;16:82.
72. Peng C, den Dekker M, Cardenas A, Rifas-Shiman SL, Gibson H, Agha G, et al. Residential proximity to major roadways at birth, DNA methylation at birth and midchildhood, and childhood cognitive test scores: project viva (Massachusetts, USA). Environ Health Perspect. 2018;126:97006.
73. Ji H, Biagini Myers JM, Brandt EB, Brokamp C, Ryan PH, Khurana Hershey GK. Air pollution, epigenetics, and asthma. Allergy Asthma Clin Immunol. 2016;12:51.
74. Sydbom A, Blomberg A, Parnia S, Stenfors N, Sandstrom T, Dahlen SE. Health effects of diesel exhaust emissions. Eur Respir J. 2001;17:733–46.
75. De Prins S, Koppen G, Jacobs G, Dons E, Van de Mieroop E, Nelen V, et al. Influence of ambient air pollution on global DNA methylation in healthy adults: a seasonal follow-up. Environ Int. 2013;59:418–24.
76. Breton CV, Salam MT, Wang X, Byun HM, Siegmund KD, Gilliland FD. Particulate matter, DNA methylation in nitric oxide synthase, and childhood respiratory disease. Environ Health Perspect. 2012;120:1320–6.
77. Commodore A, Mukherjee N, Chung D, Svendsen E, Vena J, Pearce J, et al. Frequency of heavy vehicle traffic and association with DNA methylation at age 18 years in a subset of the Isle of Wight birth cohort. Environ epigenetics. 2018;4:dvy028.
78. Tarantini L, Bonzini M, Apostoli P, Pegoraro V, Bollati V, Marinelli B, et al. Effects of particulate matter on genomic DNA methylation content and iNOS promoter methylation. Environ Health Perspect. 2009;117:217–22.
79. Salam MT, Byun HM, Lurmann F, Breton CV, Wang X, Eckel SP, et al. Genetic and epigenetic variations in inducible nitric oxide synthase promoter, particulate pollution, and exhaled nitric oxide levels in children. J Allergy Clin Immunol. 2012;129:232-9.e1–7.
80. Brunst KJ, Leung YK, Ryan PH, Khurana Hershey GK, Levin L, Ji H, et al. Forkhead box protein 3 (FOXP3) hypermethylation is associated with diesel exhaust exposure and risk for childhood asthma. J Allergy Clin Immunol. 2013;131:592-4.e1–3.
81. Somineni HK, Zhang X, Biagini Myers JM, Kovacic MB, Ulm A, Jurcak N, et al. Ten-eleven translocation 1 (TET1) methylation is associated with childhood asthma and traffic-related air pollution. J Allergy Clin Immunol. 2016;137:797–805.e5.
82. Tang WY, Levin L, Talaska G, Cheung YY, Herbstman J, Tang D, et al. Maternal exposure to polycyclic aromatic hydrocarbons and 5'-CpG methylation of interferon-gamma in cord white blood cells. Environ Health Perspect. 2012;120:1195–200.
83. Gruzieva O, Xu CJ, Breton CV, Annesi-Maesano I, Anto JM, Auffray C, et al. Epigenome-wide meta-analysis of methylation in children related to prenatal NO2 air pollution exposure. Environ Health Perspect. 2017;125:104–10.

84. Wigle DT, Arbuckle TE, Turner MC, Berube A, Yang Q, Liu S, et al. Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J Toxicol Environ Health B Crit Rev. 2008;11:373–517.
85. Windham G, Fenster L. Environmental contaminants and pregnancy outcomes. Fertil Steril. 2008;89:e111–6; discussion e7.
86. Karmaus W, Osuch JR, Eneli I, Mudd LM, Zhang J, Mikucki D, et al. Maternal levels of dichlorodiphenyl-dichloroethylene (DDE) may increase weight and body mass index in adult female offspring. Occup Environ Med. 2009;66:143–9.
87. Hansen S, Strom M, Olsen SF, Maslova E, Rantakokko P, Kiviranta H, et al. 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. 2014;122:93–9.
88. Guerrero-Preston R, Goldman LR, Brebi-Mieville P, Ili-Gangas C, Lebron C, Witter FR, et al. Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. Epigenetics. 2010;5:539–46.
89. Huen K, Yousefi P, Bradman A, Yan L, Harley KG, Kogut K, et al. Effects of age, sex, and persistent organic pollutants on DNA methylation in children. Environ Mol Mutagen. 2014;55:209–22.
90. Dao T, Hong X, Wang X, Tang WY. Aberrant 5'-CpG methylation of cord blood TNFalpha associated with maternal exposure to polybrominated diphenyl ethers. PLoS One. 2015;10:e0138815.
91. Quansah R, Armah FA, Essumang DK, Luginaah I, Clarke E, Marfoh K, et al. Association of arsenic with adverse pregnancy outcomes/infant mortality: a systematic review and meta-analysis. Environ Health Perspect. 2015;123:412–21.
92. Pacchierotti F, Spano M. Environmental impact on DNA methylation in the germline: state of the art and gaps of knowledge. Biomed Res Int. 2015;2015:123484.
93. Kaushal A, Zhang H, Karmaus WJJ, Everson TM, Marsit CJ, Karagas MR, et al. Genome-wide DNA methylation at birth in relation to in utero arsenic exposure and the associated health in later life. Environ Health. 2017;16:50.
94. Broberg K, Ahmed S, Engstrom K, Hossain MB, Jurkovic Mlakar S, Bottai M, et al. Arsenic exposure in early pregnancy alters genome-wide DNA methylation in cord blood, particularly in boys. J Dev Orig Health Dis. 2014;5:288–98.
95. Rojas D, Rager JE, Smeester L, Bailey KA, Drobna Z, Rubio-Andrade M, et al. Prenatal arsenic exposure and the epigenome: identifying sites of 5-methylcytosine alterations that predict functional changes in gene expression in newborn cord blood and subsequent birth outcomes. Toxicol Sci. 2015;143:97–106.
96. Koestler DC, Avissar-Whiting M, Houseman EA, Karagas MR, Marsit CJ. Differential DNA methylation in umbilical cord blood of infants exposed to low levels of arsenic in utero. Environ Health Perspect. 2013;121:971–7.
97. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 2008;105:17046–9.
98. Tobi EW, Lumey LH, Talens RP, Kremer D, Putter H, Stein AD, et al. DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. Hum Mol Genet. 2009;18:4046–53.
99. Marjonen H, Sierra A, Nyman A, Rogojin V, Grohn O, Linden AM, et al. Early maternal alcohol consumption alters hippocampal DNA methylation, gene expression and volume in a mouse model. PLoS One. 2015;10:e0124931.
100. Fransquet PD, et al. Perinatal maternal alcohol consumption and methylation of the dopamine receptor DRD4 in the offspring: the Triple B study. Environ Epigenet. 2016;2:dvw023.
101. Ryan J, Mansell T, Fransquet P, Saffery R. Does maternal mental Well-being in pregnancy impact the early human epigenome? Epigenomics. 2017;9:313–32.

102. Stonawski V, Frey S, Golub Y, Moll GH, Heinrich H, Eichler A. [Epigenetic modifications in children associated with maternal emotional stress during pregnancy]. Z Kinder Jugendpsychiatr Psychother. 2018;46:155–67.
103. Chatterton Z, Hartley BJ, Seok MH, Mendelev N, Chen S, Milekic M, et al. In utero exposure to maternal smoking is associated with DNA methylation alterations and reduced neuronal content in the developing fetal brain. Epigenetics Chromatin. 2017;10:4.
104. Knopik VS, Maccani MA, Francazio S, McGeary JE. The epigenetics of maternal cigarette smoking during pregnancy and effects on child development. Dev Psychopathol. 2012;24:1377–90.
105. Ligthart S, Steenaard RV, Peters MJ, van Meurs JB, Sijbrands EJ, Uitterlinden AG, et al. Tobacco smoking is associated with DNA methylation of diabetes susceptibility genes. Diabetologia. 2016;59:998–1006.
106. Mansfield AS, Wang L, Cunningham JM, Jen J, Kolbert CP, Sun Z, et al. DNA methylation and RNA expression profiles in lung adenocarcinomas of never-smokers. Cancer Genet. 2015;208:253–60.
107. Grunert M, Dorn C, Cui H, Dunkel I, Schulz K, Schoenhals S, et al. Comparative DNA methylation and gene expression analysis identifies novel genes for structural congenital heart diseases. Cardiovasc Res. 2016;112:464–77.
108. Radhakrishna U, Albayrak S, Alpay-Savasan Z, Zeb A, Turkoglu O, Sobolewski P, et al. Genome-wide DNA methylation analysis and epigenetic variations associated with congenital aortic valve stenosis (AVS). PLoS One. 2016;11:e0154010.
109. Chung VY, Tan TZ, Huang RL, Lai HC, Huang RY. Loss of discoidin domain receptor 1 (DDR1) via CpG methylation during EMT in epithelial ovarian cancer. Gene. 2017;635:9.
110. Li Z, Heng J, Yan J, Guo X, Tang L, Chen M, et al. Integrated analysis of gene expression and methylation profiles of 48 candidate genes in breast cancer patients. Breast Cancer Res Treat. 2016;160:371–83.
111. Wolf C, Garding A, Filarsky K, Bahlo J, Robrecht S, Becker N, et al. NFATC1 activation by DNA hypomethylation in chronic lymphocytic leukemia correlates with clinical staging and can be inhibited by ibrutinib. Int J Cancer. 2018;142:322–33.
112. Khongsti S, Lamare FA, Shunyu NB, Ghosh S, Maitra A, Ghosh S. Whole genome DNA methylation profiling of oral cancer in ethnic population of Meghalaya, North East India reveals novel genes. Genomics. 2018;110:112–23.
113. Acs O, Peterfia B, Hollosi P, Luczay A, Torok D, Szabo A. Methylation status of CYP27B1 and IGF2 correlate to BMI SDS in children with obesity. Obes Facts. 2017;10:353–62.
114. Dunstan J, Bressler JP, Moran TH, Pollak JS, Hirsch AG, Bailey-Davis L, et al. Associations of LEP, CRH, ICAM-1, and LINE-1 methylation, measured in saliva, with waist circumference, body mass index, and percent body fat in mid-childhood. Clin Epigenetics. 2017;9:29.
115. Janjanam VD, Mukherjee N, Lockett GA, Rezwan FI, Kurukulaaratchy R, Mitchell F, et al. Tetanus vaccination is associated with differential DNA-methylation: reduces the risk of asthma in adolescence. Vaccine. 2016;34:6493–501.
116. Berni Canani R, Paparo L, Nocerino R, Cosenza L, Pezzella V, Di Costanzo M, et al. Differences in DNA methylation profile of Th1 and Th2 cytokine genes are associated with tolerance acquisition in children with IgE-mediated cow's milk allergy. Clin Epigenetics. 2015;7:38.
117. Kim EG, Shin HJ, Lee CG, Park HY, Kim YK, Park HW, et al. DNA methylation and not allelic variation regulates STAT6 expression in human T cells. Clin Exp Med. 2010;10:143–52.
118. Luo Y, Zhou B, Zhao M, Tang J, Lu Q. Promoter demethylation contributes to TSLP overexpression in skin lesions of patients with atopic dermatitis. Clin Exp Dermatol. 2014;39:48–53.
119. Zhang C, Li J, Huang T, Duan S, Dai D, Jiang D, et al. Meta-analysis of DNA methylation biomarkers in hepatocellular carcinoma. Oncotarget. 2016;7:81255–67.
120. Li J, Huang Q, Zeng F, Li W, He Z, Chen W, et al. The prognostic value of global DNA hypomethylation in cancer: a meta-analysis. PLoS One. 2014;9:e106290.

121. Liang L, Willis-Owen SAG, Laprise C, Wong KCC, Davies GA, Hudson TJ, et al. An epigenome-wide association study of total serum immunoglobulin E concentration. Nature. 2015;520:670–4.
122. Plusquin M, Guida F, Polidoro S, Vermeulen R, Raaschou-Nielsen O, Campanella G, et al. DNA methylation and exposure to ambient air pollution in two prospective cohorts. Environ Int. 2017;108:127–36.
123. Wang IJ, Karmaus WJ, Chen SL, Holloway JW, Ewart S. Effects of phthalate exposure on asthma may be mediated through alterations in DNA methylation. Clin Epigenetics. 2015;7:27.
124. Guthikonda K, Zhang H, Nolan VG, Soto-Ramirez N, Ziyab AH, Ewart S, et al. Oral contraceptives modify the effect of GATA3 polymorphisms on the risk of asthma at the age of 18 years via DNA methylation. Clin Epigenetics. 2014;6:17.
125. Bollen KA. Structural equations with latent variables. Wiley: New York; 1989.
126. Muthen B, Asparouhov T, Rebollo I. Advances in behavioral genetics modeling using Mplus: applications of factor mixture modeling to twin data. Twin Res Hum Genet. 2006;9:313–24.
127. Muthén L, Muthén B, editors. Mplus user's guide. 6th ed. Los Angeles, CA: Muthén & Muthén; 2010.
128. Eichler EE, Flint J, Gibson G, Kong A, Leal SM, Moore JH, et al. Missing heritability and strategies for finding the underlying causes of complex disease. Nat Rev Genet. 2010;11:446–50.
129. Slatkin M. Epigenetic inheritance and the missing heritability problem. Genetics. 2009;182:845–50.
130. Felix JF, Joubert BR, Baccarelli AA, Sharp GC, Almqvist C, Annesi-Maesano I, et al. Cohort profile: pregnancy and childhood epigenetics (PACE) consortium. Int J Epidemiol. 2018;47:22–3u.
131. Kishi R, Araki A, Minatoya M, Itoh S, Goudarzi H, Miyashita C. Birth cohorts in Asia: the importance, advantages, and disadvantages of different-sized cohorts. Sci Total Environ. 2018;615:1143–54.
132. Kishi R, Araki A, Miyashita C, Itoh S, Minatoya M, Kobayashi S, et al. [Importance of two birth cohorts (n=20,926 and n=514): 15 years' experience of the Hokkaido study on environment and Children's health: malformation, development and allergy]. Nihon Eiseigaku Zasshi. 2018;73:164–77.
133. Gehring U, Casas M, Brunekreef B, Bergstrom A, Bonde JP, Botton J, et al. Environmental exposure assessment in European birth cohorts: results from the ENRIECO project. Environ Health. 2013;12:8.
134. Houseley J, Hill CS, Rugg-Gunn PJ. Annual meeting of the EpiGeneSys Network of Excellence—advancing epigenetics towards systems biology. BioEssays. 2015;37:592–5.
135. Krieger N, Davey Smith G. The tale wagged by the DAG: broadening the scope of causal inference and explanation for epidemiology. Int J Epidemiol. 2016;45:1787–808.
136. Richiardi L, Bellocco R, Zugna D. Mediation analysis in epidemiology: methods, interpretation and bias. Int J Epidemiol. 2013;42:1511–9.
137. Zhang H, Zheng Y, Zhang Z, Gao T, Joyce B, Yoon G, et al. Estimating and testing high-dimensional mediation effects in epigenetic studies. Bioinformatics. 2016;32:3150–4.
138. Arshad SH, Holloway JW, Karmaus W, Zhang H, Ewart S, Mansfield L, et al. Cohort profile: the Isle of Wight whole population birth cohort (IOWBC). Int J Epidemiol. 2018;47:1043.
139. Nagin DS, Jones BL, Passos VL, Tremblay RE. Group-based multi-trajectory modeling. Stat Methods Med Res. 2018;27:2015–23.
140. Schubeler D. ESCI award lecture: regulation, function and biomarker potential of DNA methylation. Eur J Clin Investig. 2015;45:288–93.

Chapter 21
From Research to Intervention

Philippe Grandjean

Abstract The topic of this book is of great significance to public health and should garner the attention of decision-makers. Beyond the importance of the book itself, scientists studying adverse impacts on the next generation have a responsibility to inform and interpret new findings and communicate them in the light of other relevant information, i.e., what is known and what is not (yet) known. One obstacle is that media reports may sometimes involve poor or misinterpreted science, and the public, and even research colleagues, may have difficulty separating the good from the bad. Even under ideal circumstances, good science does not automatically lead to decisions on prevention or other appropriate intervention. Oftentimes, the research generates new information only on a small aspect of a larger problem. Even if the new research documents important advantages of specific preventative interventions, other issues may well be at stake and may block immediate action. This chapter will discuss how research can better contribute to inspiring prudent decisions on prevention.

Keywords Access to information · Data interpretation · Environmental policy Health communication · Health policy · Public information · Risk assessment

The research process has not been completed once the academic reporting has taken place. In the field of developmental toxicology, there are important societal interests in the outcome of the study and its possible implications. The content of public communication on the science must necessarily involve careful interpretation of research data that may be incomplete, may involve uncertainties, and may not be easily extrapolated. Misunderstandings of the research findings can easily obfuscate

P. Grandjean (✉)
Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, MA, USA

Department of Environmental Medicine, University of Southern Denmark, Odense, Denmark
e-mail: pgrand@hsph.harvard.edu; pgrand@sdu.dk

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_21

the necessary discussion on the urgency of possible protection against environmental hazards. In particular when communicating research on risks to children from early-life exposures to environmental chemicals, researchers have an obligation to interpret the evidence responsibly. That means that the data, whether entirely new or accumulated over time, must be carefully considered in regard to its quality and relevance to the public health issue. The potential for adverse health impacts must be judged in the perspective of other relevant knowledge, and uncertainties must be carefully considered. Any conflict of interest must be declared, and its possible role minimized. The public information should explain whether the results originate from pilot data or preliminary findings, whether the results have been peer reviewed, the availability of the findings, and whether the results are new and original rather than a replication. Some trimming of details may be needed, and the timing of the communication or press release can be important.

These are straightforward issues that any university press office will emphasize, while at the same time trying to buttress the recognition of the institution as a source of highly relevant, high-quality, and trustworthy science-based information. Scientists should do so too. In fact, an old definition of an expert is an individual who, due to education and experience, can be trusted to speculate responsibly. In other words, to extrapolate from the narrow study circumstances to the larger perspective of public health. In regard to developmental exposures to toxic chemicals, this type of expertise and advice is particularly needed, perhaps most clearly regarding studies on risks involving children.

21.1 Validity and Stability of Science

When considering the validity of research, at least two major aspects deserve attention: the focus or coverage of the research and the interpretation of the results obtained [1, 2]. More specifically, the first question is what was the a priori hypothesis and how was it explored? Then follows the interpretation and possible extrapolation from the findings.

The scientific evidence must always be considered in light of both strengths and weaknesses, while also taking into account information from other disciplines. A methodologic failure may appear to weaken the support for a particular association, but the mere occurrence of some weakness, such as incomplete data, does not prove the opposite, i.e., the absence of a risk. In the past, unfortunate and erroneous rejection of early warning signals has occurred because of uncertainties or presumed confounding or other biases [3, 4]. Like risk assessment, science communication should aim at preventing both exaggeration and understatement, and this is a difficult balance.

A common concern is that the research is not appropriately addressing the question posed (the a priori hypothesis) and does not discuss the findings in a proper perspective. Perhaps affecting as much as half of biomedical research publications—such limitations have been dubbed research waste [5]. Undoubtedly, waste

also exists within the research on developmental toxicity of environmental pollutants, and certainly within environmental epidemiology [2]. Clearly, wasteful research is of little use to policy-decisions and to scientific understanding.

Further, much research tends to be repetitive (or "safe"), rather than original (and "risky"), but both replication and innovation are of course needed. This issue is of particular concern regarding environmental chemicals, where a substantial number of chemical hazards remain untested. The focus of research in this field should therefore include lesser-known hazards that could represent serious threats to human health, rather than just continuing to focus on well-characterized chemicals. However, exploring toxicants that are poorly known can be problematic in the absence of standard chemical analyses or prior knowledge on solubility, stability, binding properties, and target organs. Unfortunately, environmental health research seems not to be as innovative as one might hope. Thus, a bibliometric study of toxicology and public health journals showed that articles published during the first decade of this millennium primarily addressed chemicals that had already been well studied, and that the top-10 substances were all metals [6]. This finding suggests that environmental health research suffers from too much inertia. On the other hand, the research probably generates scientific publications at a desirable pace while utilizing existing resources.

An important reason for the inertia relates to the science paradigm that requires replication and verification to justify solid conclusions. In support of this tradition, many preliminary findings that were highly publicized later proved to be wrong [7]. As a result, both funding agencies [8] and journals [9] have announced their intention of increasing reproducibility of research.

The demand for replication is probably also fueled by the fear of erroneous or misleading research, or outright fraud. In short, can science be trusted? While fraud does occur, it is exceedingly rare, but in public perception, it is a major concern. For this reason, the backfiring may be more severe that the mere retraction of some journal articles that were found to have been plagiarized or manipulated. As an example, the search for prenatal origins of Parkinson's disease initially discovered in a mouse model how certain pesticides weakened the development of the dopamine system in the brain and thereby generate susceptibility to the disease [10]. While this approach was highly plausible and has later been supported, the research was tarnished by the discovery that cell count data used in two publications had been falsified by a young researcher [11]. Research on stem cells or fetal tissue has also been marred by cases of falsification that has produced massive condemnation and calls for limiting science in these fields. Thus, the adverse impacts of individual cases of scientific misconduct can be far reaching. When the weekly magazine The Economist ran a survey on the internet, a majority of readers chose "no" to the question whether science was to be trusted. This outcome and a more general lack of trust in science may also relate to the democratization of expertise on the internet, as I shall discuss later in this chapter.

While the wish for replication is understandable, from an innovation point of view, it is inertia-inducing and potentially counterproductive. The opposite strategy, e.g., targeting new potential hazards and lesser-known toxicants, would appear

much more attractive. Given the large number of suspected environmental hazards that have not been properly studied, the perspective of burdening science with sheer replication will therefore come at a cost.

Stability is a related, though sometimes ignored issue [12]. Could it be that the new science is a fluke and might be wiped away in the near future? This concern is not just a matter of the science being valid and solid, even if replicated. Rather, does the new evidence fit into the pattern of other results and our general understanding how potentially toxic chemicals might affect early development? Policy makers and investors cannot afford to adopt research conclusions that are unstable and steer attention into a dead-end street. Thus, if we are to take research findings on developmental toxicity seriously, then we need to be convinced that it makes sense to authorize action in this realm, perhaps by supporting both research and intervention. Accordingly, replication and verification need to be considered beyond the individual study.

The opposite consideration is also a concern, i.e., can we believe from the available evidence (or lack thereof) that there is no risk at all? A US National Research Council (NRC) committee called attention to the erroneous inference that chemicals are regarded inert or safe, unless proven otherwise [13]. Thus, inconclusive studies have been labelled "negative" or were thought to represent "no risk" rather than "uncertain information" [2]. The NRC called this tendency the "untested-chemical assumption"—that the lack of documentation means that no regulatory action is required [13]. This tradition has resulted in providing exposure limits only for a small proportion of environmental chemicals—limits that have been found to be much too high to adequately protect against adverse health effects [14]. For example, current limits for perfluorinated compounds in drinking water do not protect against the immunotoxic effects in children and may be more than 100-fold too high [15]. Thus, when scientific evidence is considered incomplete, exposure standards tend to be more lenient and prevention is deferred.

21.2 Precautionary Interpretation

Interpretation is a matter of judging the weight and validity of the observations, and guidelines exist for judging key aspects, such as statistical significance. However, it can be misleading when a 5% significance limit is strictly applied, so that a greater emphasis is placed on results that have a p value of, say, 4.9% than on results with one of 5.1% [16]. There is of course no meaningful difference between outcomes with these similar p values. Some scientists and some scientific journals oppose the reliance on p values [17, 18]. Instead of calculating whether the point estimate is "significantly" different from no effect, the confidence interval is preferred [19] (Fig. 21.1). As an additional problem, the results are often compared with a null hypothesis that the exposure has no effect, although the absence of an effect may well be unrealistic or obviously wrong, i.e., that lead exposure is not neurotoxic. A possible solution is to test the results against a plausible alternative hypothesis [20].

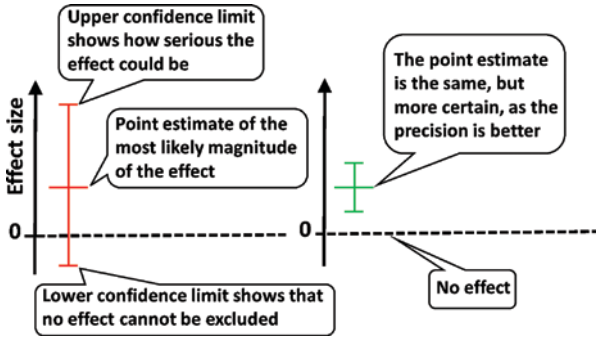


Fig. 21.1 Two studies show the same average effect (horizontal line), but the vertical line suggests that the study on the left has a larger confidence interval and more uncertainty, so that it is both in accordance with no effect (it includes zero and is therefore not statistically significant), but it also cannot exclude a large effect. The study on the right shows the same effect, now statistically significant, but due to the greater precision, this study can exclude the presence of a large effect. From Grandjean [1]. © EEA, Copenhagen, 2013

In these considerations, the Precautionary Principle (PP) may provide useful guidance [21]. Originally proposed under the United Nations auspices and later integrated in the EU treaty, the key element of the PP is the justification for appropriate public health action in response to limited (but plausible and credible) evidence of likely and substantial harm. In other words, better safe than sorry [22]. The PP is thereby aimed at avoiding possible future harm associated with suspected, but not conclusive, environmental risks. In placing importance on imperfect evidence, the PP has implications for quality judgments of research in fields, such as adverse effects of developmental toxicant exposures.

The PP offers a different approach to interpreting *p* values and confidence intervals [1]. The two studies illustrated in Fig. 21.1 show the same average effect, though with different degrees of certainty. The study on the right shows an effect that is statistically significant, as the no-effect hypothesis can be excluded. The study on the left has less precision, perhaps because it is smaller, and the point estimate does not deviate significantly from no effect (the null hypothesis). However, the upper confidence limit suggests that the study cannot exclude a large effect. In contrast, the significant study on the right would speak against the hazard of being very large. Both of these perspectives are relevant, for both studies. A focus on the upper confidence limit would have the additional advantage that it would inspire larger studies with greater precision.

From a precautionary viewpoint, the use of confidence intervals, rather than *p* values, is highly attractive. Instead of concluding that we are not sure that there is an effect at all, we can now also say that the results do not contradict an effect that might well be up to a particular magnitude. If the study is large, and when results from two or more studies are combined, the confidence interval will be narrower and reflect less statistical uncertainty. Still, from a traditional "frequentist" viewpoint, the small study cannot reject the null hypothesis and therefore does not call

for any further attention, which is in contrast to the PP view. However, both are useful, and both agree that a narrow focus on *p* values should be avoided [23].

Traditionally, science has aimed at providing the best possible evidence, but this purpose may inspire numerous replications and thereby also a delay in agreeing on a firm conclusion. In contrast, the PP refers to the particular situations of potentially serious or irreversible threats to health or to the environment, where the need to act to reduce potential hazards—before there is strong proof of harm—should take into account likely present and future costs and benefits of both action and inaction [24]. Thus, preliminary, but reliable evidence may be sufficient to justify a preliminary intervention to avoid a health hazard that could otherwise lead to serious repercussions. Thus, when new scientific evidence emerges, it needs to be considered in regard to its validity, but also in light of the perspective that may involve serious health risks from prenatal exposures. Accordingly, the PP does not inspire repetitive verification, as less than a complete proof can be considered sufficient. Further, the PP does not demand testing of a null hypothesis that an exposure may be without a discernible effect. Rather, information is requested on whether an exposure might be a serious endangerment to health. Although the PP has not been universally welcomed [25], the common sense inherent in the PP should lead to more virtuous and prudent ways of planning, conducting, reporting, and utilizing research in environmental epidemiology and in studies on developmental toxicology.

21.3 Conflicts of Interest, Skepticism, and Doubt

While ideal science can be characterized as disinterested [26], vested interests may make research less neutral and less reliable and may attempt to disprove conclusions that for some reason are unwelcome. The strategies used by private-sector actors are best illustrated by known actions taken by international tobacco companies [27, 28]. Although the industry knew about the adverse effects, they chose to invent a debate about the published science and to claim that more research was needed, while in the meantime continuing to market the hazardous product as if it was completely safe. The success of this approach shows that it can be more effective to influence the science than it is to debate the policies. The same playbook has been used by numerous other industries that have raised doubt about the scientific evidence by a multitude of methods [29].

As an example, an international call for guidelines on Good Epidemiological Practice appeared to serve a useful educational purpose. However, this “sound science” movement was not an indigenous effort from within the profession to improve the quality of scientific discourse. It turned out to be part of a sophisticated public relations strategy controlled by industry executives and lawyers whose aim was to manipulate the standards of scientific proof to serve the corporate interests of their clients [30]. Thus, strict interpretation of epidemiological rules had the purpose of dismissing epidemiological findings that, for business reasons, had to be challenged. The immediate purpose at the time was to invalidate evidence on environmental

tobacco smoke exposure, but it soon dealt with other environmental exposures. The efforts were co-funded by the Chemical Manufacturers Association (later renamed American Chemical Council) and the Philip Morris Company [30]. As a result, the battle has moved from policy discussions into the academic sphere, where an exaggerated discussion on quality and validity of the science found a fertile ground.

In parallel, efforts are sometimes made to amplify or to manufacture uncertainties with the aim of explaining away a statistically significant association [31]. Exposure imprecision is often alleged to cause exaggerated or misleading associations [32], while most often the opposite is true [33]. In addition, misleading calls may refer to the need for a Bonferroni adjustment [29] or otherwise sowing doubt about health risks [34]. Unwanted results are then criticized as "junk science."

A further strategy is to engage with willing scientists, i.e., what is known as "science for hire" or in the form of some type of "friendly" research sponsorship. When the results are published, a sponsor's interests can color the conclusions drawn. Well-known examples include studies supported by the pharmaceutical industry that were much more likely to conclude that a drug is efficacious than studies conducted without such support [35]; similar tendencies have been observed in other fields [36]. Researchers publishing results that are at odds with vested interests can become targets of unreasonable criticism and intimidation with the aim of suppressing or throwing suspicion on the unwelcome research information, as in the case of lead [37, 38] and many other environmental chemicals [39].

When adding other strategies, such as suppression of information, withholding of evidence, lambasting of whistle-blowers, and releasing of half-truths or untruths [40], a clear picture emerges of interference with science as a marketing tool, where formal declarations of conflicts of interest are oftentimes of doubtful value [41].

Skepticism toward new research findings is not limited to financial interests but can well occur in regulatory agencies that may find new research conclusions unwanted if they challenge current regulatory procedures and may require cumbersome changes that can require lengthy review of established practices and discussions on alternatives. Thus, a recent initiative in the USA to limit the research that can be applied to decisions on regulations appears to serve policy purposes rather than securing the best possible documentation [42].

Still, any researcher has a dream of discovering something new and important, and journal editors like to flash results that can raise attention. Indeed, there is a well-documented risk that frequently cited conclusions published in major journals are later found to be wrong [7].

However, the instances where misleading exaggerations occur are comparatively rare and play a much less role than the above efforts to undermine science. Further, for environmental health hazards, the main problem is rather the lack of information on the great majority of potential health risks which, due to the incompleteness or lack of evidence, are thought to be innocuous.

Due to the potential controversies and unpleasant criticism, perhaps even collegial envy, there is a much more prevalent and important bias in academic research—the downplaying of conclusions and implications. Thus, researchers frequently choose to hedge their conclusions by repeated use of words, such as "maybe,"

"perhaps," "in theory," and similar terms [43]. By softening the conclusions and avoiding attribution of specific causality and the possible policy implications, the researchers protect themselves against critique by appearing well-balanced, unassuming, and even skeptical toward one's own findings. However, this strategy has a downside. To the lay reader (or the biased investor), who is not familiar with the traditions of scientific writing, the caveats and reservations may sound like the new results really do not prove anything, and that we are still left with the same uncertainty and no justification for any intervention. To readers with a vested interest, the soft wording can be exploited through selective quotation and by emphasizing real or alleged weaknesses [44].

This tendency goes beyond one's own writings and affects peer review and evaluations of manuscripts and applications. Many researchers have had the experience that their most innovative publications had to undergo repeated reviews and revisions before being accepted for publication. Although skepticism is in the nature of science, a malignant form is the one that is veiled and expressed in terms of need for replication or emphasis of limitations of new observations, but is due to concerns about the implications, sometimes inspired by a conflict of interest. Evidence of disbelief of this kind can be difficult to track down, but one must suspect ulterior motives when environmental epidemiology research is being accused of being paparazzi science that leads to chemophobia [45].

21.4 Science and the Media

With the above issues in mind, it is no wonder that the public is confused, and that research is not easily translated to policy. Adding to the confusion, journalists often report on science the way that politics are portrayed—i.e., that we need to hear the other side, as if science was subjective and a matter to be decided by democratic vote [46]. A most relevant topic that has been contaminated by unjustified views "from the other side" is climate change, where vested interests have successfully promoted their own business interests [47].

Given this tendency, scientists have a responsibility to be clear and not bury the lede by emphasizing less relevant circumstances instead of the main message. Likewise, hedging will for sure result in the message being lost or misinterpreted.

As a modern complication, the media landscape has completely changed and will continue to change, as communication is accessible to everybody and thereby has become vulnerable to manipulation [46]. An important attribute is that search machines will prioritize sites that have attracted many hits, whether or not the information is true. Large numbers of hits may have been promoted by tweets or other internet messages that may claim to expose government mismanagement, scientific fraud, or some other attention-raising controversy that can easily become viral. An important example is the misinformation on vaccination risks that have inspired many parents to refuse having their children immunized against measles and other infections. However, vaccinations form a crucial part of modern prevention, and the

World Health Organization has calculated that immunizations save about 2–3 million lives every year and that an additional 1.5 million deaths could be prevented if global coverage could be improved (https://www.who.int/features/factfiles/immunization/en/). This example is particularly relevant, as it refers to the health and safety of the next generation, just like this book, albeit relying on lacking or misinterpreted evidence.

There is always a risk that widely available scientific information will be interpreted out of context, as seen in the anti-vaccination movement. However, what is new is the hostility against established knowledge that is counter to some preexisting beliefs, some of which may have been nurtured by outdated or refuted research findings [46]. This movement insists on autonomy and freedom to choose, rather than being told by elitist scientists what to believe in. This tendency can be fueled by well-known politicians and other notabilities who, without having any scientific expertise, have expressed the need to protect children's health and therefore support the anti-vaccination movement. Thus, the internet allows us to believe that we are all competent, we are all peers, although this is a form of misguided egalitarianism that harms the beneficial use of science [46].

This situation is not improved when journalists prefer to entertain readers for the sake of media promotion, rather than inform and educate. Controversy, whether real or not, is much greater entertainment than telling the straight story about chemical pollutants that harm babies' development. The same phenomenon affects the internet, where stories that are surprising or counter to common belief have a greater chance of being forwarded. Once they are forwarded in sufficient numbers, a Google search will show these sites on top of the search.

21.5 The Science Response

Science is often looked upon as the source of documentation, the inspiration for improvements in society, and the origin of useful innovations. Particularly in the field of environmental health, the research can be the source of controversy. Although controversy may be a necessary component of any change and improvement in society, it would seem that justified protection of the next generation's health would be better served with less controversy.

As has been described in this chapter, and as shown in Fig. 21.2, only research of high quality and stability should have an impact on appropriate interventions, and the results need to be interpreted prudently and in light of the PP. In the presence of a multitude of chemical hazards that may irreversibly impact the health of the next generation, what is needed is not just more research, but better, more targeted research that more convincingly addresses public health issues. There is much room for improvement, e.g., in our choice of research topics. Thousands of scientific articles have been published on metals present in environmental pollution, and on lead alone, about one thousand new articles are published every year. Innovative and more risk-taking research is clearly needed.

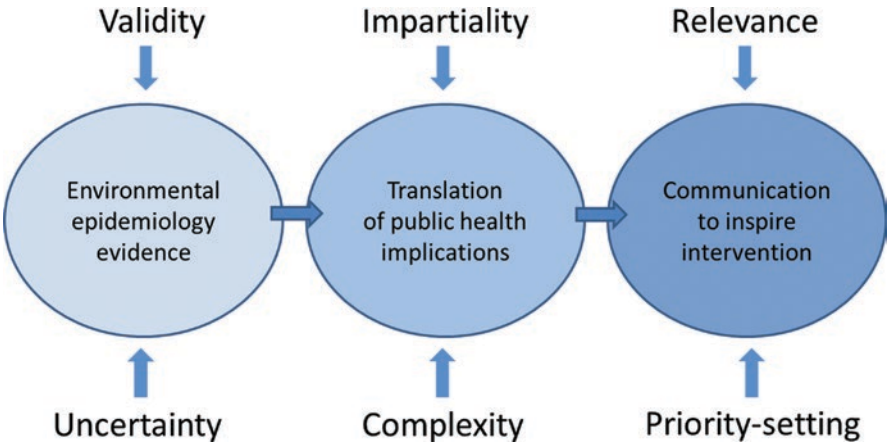


Fig. 21.2 Research findings in environmental health research must be interpreted in the light of strengths and weaknesses and communicated in respect of other relevant issues

While scientists must always acknowledge the limitations of the research evidence, the PP also suggests a consideration how much could possibly be known at this time, given the type and extent of evidence available. Noisy studies (e.g., with imprecise estimates of the causative exposure and insensitive and nonspecific outcome measures) are unlikely to detect anything but the most serious risks. These studies do not support safety claims, and the fact that the null hypothesis could not be rejected with confidence is irrelevant.

The key is that high-quality science must be linked to solid and convincing communication. Original and trustworthy data are vital in muting conspiracy theories, as they appear on the internet. Such misinformation should not deter scientists from communicating and educating, much less so in the much-needed quest to characterize chemical hazards that may adversely affect the next generation.

No matter the source of the controversy or misinformation, the basic problem is that prevention has too often been deferred due in part to the alleged absence of convincing scientific evidence. This error is recognized only when decisive evidence has finally been gathered, and it is realized that action should have been initiated much earlier on. With time, nearly all exposure limits for hazardous agents have decreased once new evidence was perceived to document harm occurring at lower exposures than previously believed [14]. Countless examples exist that the costs of such optimistic inaction are huge [3]. In other words, by being skeptical and doubtful about developmental toxicity, we are taking the next generation hostage. As happened with lead, the hesitation to regulate the useful addition of lead compounds to gasoline caused immense suffering and loss of brain functions in a whole generation of children [37].

Science cannot by itself provide the full support or guideline for prudent decision-making on environmental hazards. But science can provide documentation, and both the quality and the relevance of the research can be improved. Moreover, through balanced communication, research can inspire more adequate protection of the next generation against health hazards.

References

1. Grandjean P. Science for precautionary decision-making. In: Gee D, Grandjean P, Hansen SF, van den Hove S, MacGarvin M, Martin J, Nielsen G, Quist D, Stanners D, editors. Late lessons from early warnings. II. Copenhagen: European Environment Agency; 2013. p. 517–35.
2. Grandjean P. Seven deadly sins of environmental epidemiology and the virtues of precaution. Epidemiology. 2008;19(1):158–62.
3. Gee D. Late lessons from early warnings: toward realism and precaution with endocrine-disrupting substances. Environ Health Perspect. 2006;114(Suppl 1):152–60.
4. European Environmental Agency. Late lessons from early warnings: the precautionary principle 1896-2000. Copenhagen. Contract No.: Environmental issue report No. 22. 2001.
5. Chalmers I, Glasziou P. Avoidable waste in the production and reporting of research evidence. Lancet. 2009;374(9683):86–9.
6. Grandjean P, Eriksen ML, Ellegaard O, Wallin JA. The Matthew effect in environmental science publication: a bibliometric analysis of chemical substances in journal articles. Environ Health. 2011;10:96.
7. Ioannidis JP. Why most published research findings are false. PLoS Med. 2005;2(8):e124.
8. Collins FS, Tabak LA. Policy: NIH plans to enhance reproducibility. Nature. 2014;505(7485):612–3.
9. Journals unite for reproducibility. Nature. 2014;515(7525):7.
10. Barlow BK, Richfield EK, Cory-Slechta DA, Thiruchelvam M. A fetal risk factor for Parkinson's disease. Dev Neurosci. 2004;26(1):11–23.
11. Dunning H. Parkinson's researcher fabricated data. The Scientist [Internet]. 2012. Available from https://www.the-scientist.com/news-opinion/parkinsons-researcher-fabricated-data-40816.
12. Broadbent A. Philosophy of epidemiology. Basingstoke/New York: Palgrave Macmillan; 2013. p. 203.
13. National Research Council. Science and decisions: advancing risk assessment. Washington, DC: National Academy Press; 2009.
14. Goldsmith JR. Perspectives on what we formerly called threshold limit values. Am J Ind Med. 1991;19(6):805–12.
15. Grandjean P, Budtz-Jorgensen E. Immunotoxicity of perfluorinated alkylates: calculation of benchmark doses based on serum concentrations in children. Environ Health. 2013;12:35.
16. Holman CD, Arnold-Reed DE, de Klerk N, McComb C, English DR. A psychometric experiment in causal inference to estimate evidential weights used by epidemiologists. Epidemiology. 2001;12(2):246–55.
17. Lang JM, Rothman KJ, Cann CI. That confounded P-value. Epidemiology. 1998;9(1):7–8.
18. Amrhein V, Greenland S, McShane B. Scientists rise up against statistical significance. Nature. 2019;567(7748):305–7.
19. Thompson WD. Statistical criteria in the interpretation of epidemiologic data. Am J Public Health. 1987;77(2):191–4.
20. Johnson VE. Revised standards for statistical evidence. Proc Natl Acad Sci U S A. 2013;110(48):19313–7.
21. Grandjean P. Toxicology research for precautionary decision-making and the role of human & experimental toxicology. Hum Exp Toxicol. 2015;34(12):1231–7.
22. Science Communication Unit. Science for environment policy -the precautionary principle: decision-making under uncertainty (future brief). Brussels: European Commission Directorate-General Environment; 2017.
23. Stang A, Poole C, Kuss O. The ongoing tyranny of statistical significance testing in biomedical research. Eur J Epidemiol. 2010;25(4):225–30.
24. Grandjean P. Implications of the precautionary principle for primary prevention and research. Annu Rev Public Health. 2004;25:199–223.
25. Stirling A, Gee D. Science, precaution, and practice. Public Health Rep. 2002;117(6):521–33.
26. Merton RK. The Matthew effect in science. The reward and communication systems of science are considered. Science. 1968;159(810):56–63.

27. Glantz SA. The cigarette papers. Berkeley: University of California Press; 1996. p. 539.
28. Landman A, Glantz SA. Tobacco industry efforts to undermine policy-relevant research. Am J Public Health. 2009;99(1):45–58.
29. Michaels D. Doubt is their product: how industry's assault on science threatens your health. Oxford/New York: Oxford University Press; 2008. p. 372.
30. Ong EK, Glantz SA. Constructing "sound science" and "good epidemiology": tobacco, lawyers, and public relations firms. Am J Public Health. 2001;91(11):1749–57.
31. Michaels D, Monforton C, Lurie P. Selected science: an industry campaign to undermine an OSHA hexavalent chromium standard. Environ Health. 2006;5:5.
32. Blair A, Stewart P, Lubin JH, Forastiere F. Methodological issues regarding confounding and exposure misclassification in epidemiological studies of occupational exposures. Am J Ind Med. 2007;50(3):199–207.
33. Budtz-Joergensen E, Keiding N, Grandjean P. Approaches to handling uncertainty when setting environmental exposure standards. In: Baveye P, Mysiak J, Laba M, editors. Uncertainties in environmental modeling and consequences for policy making. Dordrecht: Springer; 2009. p. 267–80.
34. Gori GB. Science, imaginable risks, and public policy: anatomy of a mirage. Regul Toxicol Pharmacol. 1996;23(3):304–11.
35. Lundh A, Lexchin J, Mintzes B, Schroll JB, Bero L. Industry sponsorship and research outcome: systematic review with meta-analysis. Intensive Care Med. 2018;44(10):1603–12.
36. Fabbri A, Lai A, Grundy Q, Bero LA. The influence of industry sponsorship on the research agenda: a scoping review. Am J Public Health. 2018;108(11):e9–e16.
37. Needleman HL. The removal of lead from gasoline: historical and personal reflections. Environ Res. 2000;84(1):20–35.
38. Markowitz GE, Rosner D. Deceit and denial : the deadly politics of industrial pollution. Berkeley: University of California Press; 2002. p. 408.
39. Krimsky S. Science in the private interest. Lanham: Rowman & Littlefield; 2003.
40. Kurland J. The heart of the precautionary principle in democracy. Public Health Rep. 2002;117(6):498–500.
41. Baur X, Budnik LT, Ruff K, Egilman DS, Lemen RA, Soskolne CL. Ethics, morality, and conflicting interests: how questionable professional integrity in some scientists supports global corporate influence in public health. Int J Occup Environ Health. 2015;21(2):172–5.
42. Ioannidis JPA. All science should inform policy and regulation. PLoS Med. 2018;15(5):e1002576.
43. Hyland K. Hedging in scientific research articles. Amsterdam: John Benjamins; 1998.
44. Grandjean P. Late insights into early origins of disease. Basic Clin Pharmacol Toxicol. 2008;102(2):94–9.
45. Safe SH. Xenoestrogens and breast cancer. N Engl J Med. 1997;337(18):1303–4.
46. Nichols TM. The death of expertise : the campaign against established knowledge and why it matters. New York: Oxford University Press; 2017. p. 252.
47. Oreskes N. Beyond the ivory tower. The scientific consensus on climate change. Science. 2004;306(5702):1686.

Part V
Closing

Chapter 22
Further Direction of Research and Policy Making of Environment and Children's Health

Reiko Kishi and Atsuko Araki

Abstract The Minamata Convention was established following the Minamata disaster. To protect humans and the environment from persistent organic pollutants, the Stockholm Convention was established. However, there are many remaining chemicals with potential hazardous properties so that we must speed up and shorten the time to take action for such chemicals. Emerging environmental hazards including toxic chemicals are a threat to child health. A considerable number of birth cohorts have been recently established, and evidence reveals that there are certain adverse associations between environmental chemical exposures in utero and adolescent and children's health. However, there are remaining agenda in preventing exposure of children to hazardous chemicals. We have pointed out challenges in future studies on (1) other risk factors that are not comprehensively addressed in this book, (2) mixture exposure, (3) genetics, (4) biomolecular approaches, (5) birth cohorts and consortiums, and (6) inclusion of environment into the original Developmental Origins of Health and Disease concept, as well as (7) long-term follow-up. The United Nations set the sustainable development goals. We should act together to create cleaner and safer environments for children by preventing exposure to chemical hazards, which will contribute to a healthier, more secure, and sustainable future for the world.

Keywords Children · Environmental chemicals · Genetics and epigenetics Exposome · Regulations · Social factors · Global collaboration · Cohort consortium · Public health intervention · Sustainable development goals

R. Kishi · A. Araki (✉)
Hokkaido University Center for Environmental and Health Sciences, WHO Collaborating Centre for Environmental Health and Prevention of Chemical Hazards, Sapporo, Japan
e-mail: AAraki@cehs.hokudai.ac.jp

R. Kishi, P. Grandjean (eds.), *Health Impacts of Developmental Exposure to Environmental Chemicals*, Current Topics in Environmental Health and Preventive Medicine, https://doi.org/10.1007/978-981-15-0520-1_22

22.1 Introduction

The World Health Organization (WHO) reported that 25% of the global burden of disease is due to environmental risks and 45% of the burden falls on children aged <5 years [1]. Traditional hazards include air pollution, water pollution, sanitation, and vector-borne diseases [1]. Emerging environmental hazards, including toxic chemicals, electronic waste (e-waste), and climate change, are another category of threat to child health [1]. Through this book, associations between adverse health outcomes on children and exposures to environmental chemicals in utero to early childhood have been introduced. A considerable number of birth cohorts have been recently established, and evitable studies have been published to fill the gap between our knowledge. However, there are remaining agenda in preventing exposure of children to hazardous chemicals. Recent remarkable innovation brings us into a new era. The development of new technologies enables us to obtain enormous information and data, which presents new challenges. In this last chapter of the book, we discuss these challenges and appropriate directions.

22.2 Challenges in the Future Studies

22.2.1 Other Risk Factors that Are Not Comprehensively Addressed in this Book

As mentioned in the Introduction, environmental risks in children highlighted by the WHO are inadequate water, sanitation and hygiene, radiation, emerging threats such as e-waste, and climate change [1]. The Lancet Commission revealed that climate change affects the health of vulnerable populations by more intensive heat waves and transmission of vector-borne diseases [2]. We have highlighted the following factors:

(i) Physical factors

For radiation, effects of low doses of radiation need to be determined after the occurrence of nuclear accidents as in Fukushima, where exposure levels via radiation contaminated foods were quite lower than that of Chernobyl [3]. According to the results of the Fukushima Health Management Survey, the prevalence of thyroid nodules, which may develop into thyroid cancer, in the Fukushima evacuation area 3–6 years after the accident was significantly higher than that in other areas [4]. The preliminary results to this important endpoint drew international attention as found in comments on letters to editor [5] and authors' responses [6]. Another ecological study reported the detection of excess thyroid cancer incidence among children and adolescents in Fukushima within 4 years of the accident [7]. However, several limitations of the study have been highlighted by other researchers including the possible overestimation which could be due to the cancer screening program, the study's ecological design, the methodology of the prevalence pool with the 4-year duration, and the lack of personal exposure doses and cancer incidence relationship [8–11].

In any case, of utmost importance is the consideration of the long-term health effects of an accident and the establishment of infrastructure to identify and follow populations to monitor their health outcomes on a regular basis, further the establishment of mechanisms to estimate individual-level radiation dose allow the assessment of meaningful dose–response analysis [12]. Meanwhile, in Fukushima, the health risks related to lifestyle and psychosocial factors should not be ignored as the accident seriously damaged so-called social capital of communities and bonds within and between communities [13].

Electromagnetic fields are also one of the most common and fastest growing environmental factors. As the frequency range of mobile phones has been shifting from time to time, it is also recommended to study the effects of exposure on children's health [14].

(ii) Biological factors and dampness

Simultaneously, we must not forget the environment other than hazardous chemicals and air pollution. For asthma and allergies, indoor environmental factors as mould and dampness are well-known factors, in addition to plasticizers used in building materials [15]. We have addressed the latest issues of these topics in the e-book entitled 'Indoor Environmental Quality and Health Risk toward Healthier Environment for All', one of the e-book series of 'Current Topics in Environmental Health and Preventive Medicine' [16].

(iii) Social factors including stress

Social factors, the conditions of daily life in which people are born, grow, work, and live, are another area to develop to improve children's health [1]. In Japan, which is generally considered as a highly developed country, the poverty rate of children aged <17 years is 14%, which is indicated as one in seven children and higher than the OECD average [17]. Low educational level and low annual income of both parents were associated with increased risk of small for gestational age in infants, through tobacco exposure, low maternal BMI before pregnancy, and alcohol intake [18]. Maternal personality trait and stress are also known factors that affect birth outcomes [19]. Thus, integration of biomedical and psychosocial approaches to reduce adverse health outcomes of children should be considered. Social determinants vary in different countries. Thus, to encourage the individual's initiative and health literacy, e.g. through education, performing a detailed analysis on the socioeconomic factors in each country including the historical background would help find a solution to this problem in each country.

22.2.2 Exposure Monitoring and Mixture Exposure of Chemicals

Human biomonitoring provides a direct measure of actual personal exposure to environmental chemicals. Many studies conducted biomonitoring using maternal and child specimens, such as blood and urine, to measure the exposure level. The sensitivity of the analytical instrument improved, and we were able to detect even lower

exposure level than before. A number of countries have long-term national biomonitoring programs, for example, the National Health and Nutrition Examination Survey in the USA (NHANES) [20], German Environmental Survey (GerES) [21], Flemish Environment and Health Study (FLEHS) [22], and Korean National Environmental Health Survey (KoNEHS) [23]. A European biomonitoring study, the Demonstration of a Study to Coordinate and Perform Human Biomonitoring on a European Scale (DEMOCOPHES), reported that 17 countries tested a common approach for human biomonitoring surveys on the European common protocol with comparable scale [24]. These biomonitoring programs have established reference values for each area, showed trends of chemical exposures over time, and determined regional differences in contaminant levels [24]. These projects that made biomonitoring data comparable are ideal.

Individuals are exposed to a wide variety of environmental factors. There are increasingly more chemicals introduced to the market, and humans are exposed to a combination of such chemicals. Most studies examine only one kind or class of chemicals. Thus, the effect of mixture exposure of chemicals on development of adverse health outcomes needs to be analysed. However, as many chemicals share exposure sources, existing multiple regression models have limit on use due to multicollinearity. As detailed in Chap. 18, further direction would be into exposome, the concept of representing all non-genetic exposures experienced during the life course. Furthermore, non-target analysis may help determine the chemicals to be identified in the biomatrix of >10,000 molecular features classified as unknown [25].

In addition to biomonitoring studies, it is worth noting the importance of studies on exposure source to reduce or prevent further exposure. In Part 2, Chaps. 10, 11, 12, 13, 14, 15, and 16 of this book describe where each chemical is used and what could be potential exposure sources. Potential major exposure sources of environmental chemicals are contaminated air, food, and water. Another source could be contaminated indoor air and dust. A market basket is one way of finding potential food contamination as an exposure source. Other studies examine the characteristics of participants highly exposed to chemicals. Other studies conduct an intervention to find if the exposure level decreased. However, such studies are still limited, especially for emerging chemicals.

22.2.3 Genetic Susceptibility

The changes or variations in DNA of individuals, for example, single nucleotide polymorphisms (SNPs), may modify the risk. In the Hokkaido Study, we have reported gene-environment interactions of maternal *SNPs* with prenatal smoking and dioxin exposure and birth size of children. When considering specific genotypes of enzymes and receptors, such as *AhR, CYP1A1, CYP2E1, GSTM1, GSTT1, MTHFR, NAT2, NQO1,* and *XRCC1*, a larger birth size reduction was noted among smokers and passive smokers with evaluated dose–response relationship with

maternal cotinine level [26–32] and maternal dioxin exposure [33] during pregnancy. The findings suggest that there are certain genetically high-risk groups in relation to environmental chemical exposures. It is unable to convert innate DNA sequence of individuals. Thus, the policy and regulation to prevent exposure to chemical hazards should be adjusted to the most vulnerable groups, considering gene-environment interaction. However, the studies on gene-environment interaction of environmental chemical exposures on child health are limited and should be further explored. Chapter 19 of this book introduced the recent rapid innovation of technologies in the analyses of genomic DNA sequence. Genome-wide association studies (GWAS) and next-generation and whole-genome sequencing in an individual become realistic. The technology of GWAS provides another theoretical use of genetic variants as proxies for environmental exposures. Mendelian randomization is a use of genetic variations as proxies for environmentally modifiable exposures in observational studies and is a powerful strategy developed in genetic epidemiology [34]. Detailed information of Mendelian randomization is written in Chap. 19 of this book.

22.2.4 Biomolecular Mechanistic Approaches

Needless to say, exposure to hazardous chemicals has been associated with adverse health outcomes. Then, what could be the underlying mechanisms between chemical exposures and adverse health outcomes? One aspect is epigenetic regulation. The stable transmission of cellular information during cell division based on gene expression levels other than that derived from the DNA sequence itself is defined as epigenetic inheritance [35]. As detailed in Chap. 20 of this book, the main types of epigenetic inheritance are DNA methylation, chromatin remodelling, genomic imprinting, and long-range control by the chromatin structure [35]. Of these, DNA methylation occurs by the addition of a methyl group to a cytosine at cytosine–guanine dinucleotide (CpG) loci and acts like a switch on gene expression [36]. It is reported that perfluorooctanoic acid (PFOA) exposure is significantly associated with the reduction of ponderal index at birth via mediation of IGF2 hypomethylation, which accounts for 21% of the total effect [37]. Furthermore, it becomes possible to determine genome-wide DNA methylation. However, studies that examine environmental chemical exposure and alteration of epigenome-wide studies are limited to methyl mercury [38], DDT, and polychlorinated biphenyls (PCBs) [38, 39], per- and polyfluoroalkyl substances [40, 41], and phthalates [42, 43]. Characterization of epigenetic changes may provide valuable insight into the mechanism by which our health is influenced by the environment and biomarkers of exposure [44]. Moreover, epigenetic modification would be a key mediator between exposure and health outcomes. Thus, Karmaus et al. mentioned in Chap. 20 that 'Once causal and molecular pathways are elucidated, epigenetic markers will serve as diagnostic and therapeutic targets facilitating the vision of personalized precision medicine'.

Genetics and epigenetics indicate information, but metabolomics and proteomics indicate function. To understand the phenomenon of chemical hazards, these functional omics studies become crucial. Moreover, a pathway-based understanding of the chemical effects has been elucidated. The Adverse Outcome Pathway framework is introduced in detail in Chap. 17. From the traditional animal toxicological data, more studies to illustrate the association between molecular initiating events to adverse organ outcomes and mechanism-specific endpoint studies are needed [45].

Although the biomolecular approach is useful in determining the mechanisms, it is not sufficient to change children's actual environmental world. To establish an effective intervention for individuals, a biosocial model with biomolecular models is necessary. It is because the lifestyle and behaviour of individuals are affected by individual's social class and education. Moreover, it is necessary to link an environmental policy of each municipality and country to prevent health risks on systemic reforms.

22.2.5 Direction of Further Birth Cohort Studies and Consortiums

After the launch of birth cohorts in different countries, a birth cohort consortium has been recently established in the area base, as in Europe (Environmental Health Risks in European Birth Cohorts: ENRIECO) and Asia (Birth Cohort Consortium of Asia: BiCCA) [46, 47]. There are also consortiums based on common techniques. The Early Growth Genetics (EGG) consortium represents a collaborative effort to combine data from multiple GWAS in order to identify additional human genome loci that have an impact on various traits related to early growth [48]. The Pregnancy And Childhood Epigenetics (PACE) consortium comprised of researchers who are interested in studying the early life environmental impacts on human disease using epigenetics [49]. The EArly Genetics and Lifecourse Epidemiology (EAGLE) consortium is a consortium on pregnancy and birth cohorts that aims to investigate the genetic basis of phenotypes in antenatal and early life and childhood [44]. The Consortium on Thyroid and Pregnancy is an international collaboration to create a formal platform for cooperation between researchers and cohorts that facilitates high-quality studies on the association of gestational thyroid function and adverse pregnancy outcomes [50]. New technologies in omics studies on international collaborations would add a new dimension to current knowledge and provide novel discovery in the future. Collaborations of as many studies as possible enable the performance of joint-data analysis and meta-analysis and increase statistical power to obtain new findings. However, the importance of using each cohort, even a small cohort, good design, and original hypothesis should be considered to obtain novel findings [51]. Without a good single cohort, no collaboration can happen.

22.2.6 *Expansion of the Environment in the Developmental Origins of Health and Disease for Prevention*

In 1986, Barker and Osmond observed a relationship between poor nutrition in early life and risk of ischemic heart disease in adulthood [52]. This observation had shown the importance of the intrauterine and early childhood nutritional environment with the risk of disease in later life. This new paradigm for the aetiology of disease is called the Developmental Origins of Health and Disease (DOHaD) hypothesis [53, 54]. Although the first concept of DOHaD focused on nutrition, it is important to expand and include environmental chemical exposures into the original DOHaD concept [55]. Under this concept, the first international conference known as Prenatal Programming and Toxicology (PPTOX) was held to convey the role of developmental exposures to environmental pollutants and disease outcomes later in life [55]. The European Union projected the concept of Environmentally-induced DOHaD (E-DOHaD), which targeted atmospheric pollutants and chemicals with endocrine-disrupting properties [56].

22.2.7 *Long-Term Follow-Up from Parental Periconceptional Periods*

The health outcomes in this book focus on children. However, it is not well known how health outcomes, such as thyroid and reproductive hormone production, body size, neurodevelopment, and onset of puberty, in early childhood affect later health outcomes. Even in relatively old cohorts established in the twentieth century, children have only reached 30 years of age so far, but in most cohorts established in the twenty-first century, participants still in their teenage years are the oldest. Moreover, the 'transgenerational' theory was established [57]. This means that exposure to environmental chemicals, such as obesogens, during pregnancy affects not only the offspring but also future generations. Thus, to determine the effect of prenatal to early life exposure, a long-term follow-up is warranted.

Another element to consider is how far in the past we have to look. In the DOHaD concept, parental environmental factors, such as diet, body composition, metabolism, and stress, affect the health and disease risk of individuals throughout their lives. The animal models of maternal and paternal mouse undernutrition in the protein meta-analysis suggest the importance of parental periconceptional contributions to postnatal outcomes [58]. Thus, the parental preparation for pregnancy should begin before conception to protect the lifetime health of the offspring [58], and studies on the preconceptional period as a crucial period should also merit attention.

22.3 Measures of Global Chemical Regulations

As mentioned in the previous paragraph, especially in Chap. 1, Japan has faced numerous environmental problems, such as mercury poisoning (Minamata disease), cadmium pollution in soil and rice (Itai-itai disease) [59], and air pollution (Yokkaichi asthma), especially after World War II. However, drastic improvement in environment-related legislation including the Basic Law for Environmental Pollution Control in 1967 was conducted. The Environment Agency was launched in 1971, and various measures were performed to prevent environmental pollution by chemical substances. Japan's domestic environmental measures have made great strides and actively contribute to the international transfer of environmental improvement technologies. Furthermore, in 2013, Japan led the adoption of the Minamata Convention on Mercury, and in 2017, ratification of countries reached 50 locations and entered into force. The Convention internationally regulates the trade of mercury-containing products with the aim of preventing global mercury pollution and health and environmental hazards.

To reduce and eliminate the release of persistent organic pollutants (POPs) in the environment, the Stockholm Convention on POPs was adopted on May 22, 2001 and entered into force on May 17, 2004 [60]. In the Stockholm Convention, each party shall prohibit production, use, import, and export of chemicals listed in the Annex. POPs require a long time to degrade, e.g. years to decades, and remain in the environment once they are disposed. The concentrations of PCBs, dioxins, and organochlorine pesticides in the human blood have been gradually decreasing [61–63]. In the Hokkaido Study, perfluorooctanesulfonate (PFOS) concentration has significantly decreased from 2003 to 2012 [64], and the regulation of PFOS through the Stockholm Convention has successfully reduced the exposure level.

However, in addition to these POPs, there are many emerging chemicals that we have been exposed to. For example, > 60,000 chemicals have been used in the industry in Japan, and additional 1500 compounds are introduced annually [65]. The numbers of registered pesticides and insecticides are 4328 and 1109, respectively, which totals to over 5000 compounds in Japan (Food and Agricultural Materials Inspection Center). Plastic waste and microplastics are becoming a major concern, as particles of plastics remain for a long period [60]. What attract attention are especially marine plastic litter on the global scale presently and harm to marine animals. We should also recognize that plastic additives, such as bisphenols, phthalates, and phosphate flame retardants, have endocrine-disrupting properties as discussed in detail in previous chapters and are released from products [66].

PFOS and PFOA have been listed in the Stockholm Convention in 2009 and 2019, respectively. The gradual decrease of PFOA and PFOA levels began years before regulation, about 2000, whereas many alternative chemicals are introduced to the market as replacement to historical POPs. Increase in those of alternatives such as perfluorononanoic acid (PFNA) and perfluorodecanoic (PFDA) acid is reported [64, 67–69]. Similarly, in January 2019, REACH expands the number of restricted phthalates to four and the scope to all plasticized materials by providing a

legal definition for 'plasticized material', 'prolonged contact with human skin', and 'childcare article' [70]. Ahead, the regulation of di(2-ethylhexyl) phthalate and bisphenol A use results in increase temporal trend of alternative chemicals such as di-iso-nonyl cyclohexane 1,2-dicarboxylate (DINCH) and bisphenol S and F [71, 72]. E-waste is released from electrical and electronic equipment and components, and residents in recycling areas are not well aware of exposure to e-waste [73]. E-waste recycling greatly occurs in the informal sector, and children can be exposed to chemicals derived from e-waste by unsafe recycling activities [73]. Children are especially vulnerable to the health risks induced by e-waste and, therefore, need more specific protection. Beyond the individual's health, to protect the land, air, and water, the global structure of industrial waste processing is critical.

As Grandjean states in Chap. 21, 'scientists have a responsibility to be clear and not bury the lead by emphasizing less relevant circumstances instead of the main message'. Through accurate communication, individuals can face the chemical hazards and risk. Scientists should also communicate with physicians, especially gynaecologists and paediatricians, to take a specific action to prevent hazardous exposure especially in prospective parents [74]. An improved public health strategy should also be considered. For regulation and legal intervention, as written in detail in Chap. 21, 'science can provide documentation in both the quality and relevant way'. It took almost two decades since the International Programme on Chemical Safety, a joint programme of various United Nations (UN) agencies, including the WHO, published the global assessment of the state-of-science of endocrine disruptors, which was updated in 2012 [75, 76]. We must speed up and shorten the time to take action.

22.4 Conclusions: No One Left Behind to Start a Healthy Life

The UN set the sustainable development goals (SDGs) as the blueprint to achieve a better and more sustainable future for all. The global challenges we face are those related to poverty, inequality, climate, environmental degradation, prosperity, and peace and justice. We have to achieve each goal and target by 2030 to leave no one behind. Environment is a key in SDGs. The primary goal of environmental epidemiological studies on children is Goal 3 (Good Health and Well-being). However, our target is not only health and well-being. Reduction in exposure to hazardous chemicals and maintenance of clean air would lead to the achievement of Goal 11 (Sustainable Cities and Communities), Goal 12 (Responsible Production and Consumption), Goal 14 (Life Below Water), and Goal 15 (Life On Land). Moreover, the measures to social factors simultaneously led to the achievement of Goal 1 (No Poverty), Goal 4 (Quality Education), Goal 5 (Gender Equality), and Goal 10 (Reduced Inequalities). Lastly, Goal 17 (Partnership) is a key to achieve our goal. We should act together to create healthier, cleaner, and safer environments for children, which will contribute to a more secure future for the world [77]. Therefore, the accumulation of the scientific knowledge and reinforcement of political actions based on scientific evidence are more important.

Acknowledgements This research was supported in part by Grants-in-Aid for Scientific Research from the Japan Ministry of Health, Labour, and Welfare; and the Japan Agency for Medical Research and Development (AMED) under Grant Number JP18gk0110032.

References

1. World Health Organization. Inheriting a sustainable world? Atlas on children's health and the environment. Brighton: WHO; 2017.
2. Watts N, Amann M, Ayeb-Karlsson S, et al. The lancet countdown on health and climate change: from 25 years of inaction to a global transformation for public health. Lancet. 2018;391(10120):581–630.
3. Kamiya K, Ozasa K, Akiba S, et al. Long-term effects of radiation exposure on health. Lancet. 2015;386(9992):469–78.
4. Akiba S, Nandakumar A, Higuchi K, Tsuji M, Uwatoko F. Thyroid nodule prevalence among young residents in the evacuation area after Fukushima Daiichi nuclear accident: results of preliminary analysis using the official data. J Radiat Cancer Res. 2017;8(4):174.
5. Reiners C, Kesminiene A, Schüz J. Comments on 'thyroid nodule prevalence among young residents in the evacuation area after Fukushima Daiichi nuclear accident: results of preliminary analysis using the official data. J Radiat Cancer Res. 2019;10(1):79–80.
6. Akiba S. Author reply. J Radiat Cancer Res. 2019;10(1):80–1.
7. Tsuda T, Tokinobu A, Yamamoto E, Suzuki E. Thyroid cancer detection by ultrasound among residents ages 18 years and younger in Fukushima, Japan: 2011 to 2014. Epidemiology. 2016;27(3):316–22.
8. Jorgensen TJ. To the Editor "Thyroid cancer among young people in Fukushima". Epidemiology. 2016;27(3):e17.
9. Takamura N. To the Editor "Thyroid cancer among young people in Fukushima". Epidemiology. 2016;27(3):e18.
10. Wakeford R, Auvinen A, Gent RN, Jacob P, Kesminiene A, Laurier D, Schuz J, Shore R, Walsh L, Zhang W. To the Editor "Thyroid cancer among young people in Fukushima". Epidemiology. 2016;27(3):e20–1.
11. Takahashi H, Ohira T, Yasumura S, Nollet KE, Ohtsuru A, Tanigawa K, Abe M, Ohto H. To the Editor "Thyroid cancer detection by ultrasound among residents ages 18 years". Epidemiology. 2016;27(3):e21.
12. Davis S. Screening for thyroid cancer after the Fukushima disaster—what do we learn from such as effort? Epidemiology. 2016;27(3):323–5.
13. Akiba S, Nandakumar A, Uwatoko F. Social and health effects of Fukushima nuclear accident on residents and evacuees. In: Mishra KP, editor. Biological responses, monitoring and protection from radiation exposure. New York: Nova Publishers, Inc.; 2015. p. 91–105.
14. Electromagnetic fields and public health: mobile phones. https://www.who.int/en/news-room/fact-sheets/detail/electromagnetic-fields-and-public-health-mobile-phones. Accessed 1 July 2019.
15. Norback D, Kishi R, Araki A, editors. Indoor environmental quality and health risk toward healthier environment for all. Singapore: Springer; 2019.
16. Boas M, Frederiksen H, Feldt-Rasmussen U, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 2010;118(10):1458–64.
17. Income inequality (indicator). https://www.oecd-ilibrary.org/social-issues-migration-health/income-inequality/indicator/english_459aa7f1-en. Accessed 17 June 2019.
18. Tamura N, Hanaoka T, Ito K, et al. Different risk factors for very low birth weight, term-small-for-gestational-age, or preterm birth in Japan. Int J Environ Res Public Health. 2018;15:369.

19. Chatzi L, Koutra K, Vassilaki M, et al. Maternal personality traits and risk of preterm birth and fetal growth restriction. Eur Psychiatry. 2013;28(4):213–8.
20. National Health and Nutrition Examination Survey. https://www.cdc.gov/nchs/nhanes/index.htm. Accessed 1 July 2019.
21. German Environmental Survey. https://www.umweltbundesamt.de/en/topics/health/assessing-environmentally-related-health-risks/german-environmental-survey-geres. Accessed 1 July 2019.
22. Schoeters G, Govarts E, Bruckers L, et al. Three cycles of human biomonitoring in Flanders—time trends observed in the Flemish environment and health study. Int J Hyg Environ Health. 2017;220(2, Part A):36–45.
23. Choi W, Kim S, Baek Y-W, Choi K, et al. Exposure to environmental chemicals among Korean adults—updates from the second Korean National Environmental Health Survey (2012–2014). Int J Hyg Environ Health. 2017;220(2, Part A):29–35.
24. http://www.eu-hbm.info/democophes. Accessed 1 July 2019.
25. Andra SS, Austin C, Patel D, Dolios G, Awawda M, Arora M. Trends in the application of high-resolution mass spectrometry for human biomonitoring: an analytical primer to studying the environmental chemical space of the human exposome. Environ Int. 2017;100:32–61.
26. Kishi R, Araki A, Miyashita C, Kobayashi S, Miura R, Minatoya M. The Hokkaido study on environment and children's health. In: Sata F, Fukuoka H, Hanson M, editors. Pre-emptive medicine: public health aspects of developmental origins of health and disease. Current topics in environmental health and preventive medicine. Singapore: Springer; 2019. p. 145–63.
27. Kobayashi S, Sata F, Sasaki S, et al. Combined effects of AHR, CYP1A1, and XRCC1 genotypes and prenatal maternal smoking on infant birth size: biomarker assessment in the Hokkaido study. Reprod Toxicol. 2016;65:295–306.
28. Kobayashi S, Sata F, Sasaki S, et al. Modification of adverse health effects of maternal active and passive smoking by genetic susceptibility: dose-dependent association of plasma cotinine with infant birth size among Japanese women—the Hokkaido study. Reprod Toxicol. 2017;74:94–103.
29. Sasaki S, Kondo T, Sata F, et al. Maternal smoking during pregnancy and genetic polymorphisms in the Ah receptor, CYP1A1 and GSTM1 affect infant birth size in Japanese subjects. Mol Hum Reprod. 2006;12(2):77–83.
30. Asaki S, Sata F, Katoh S, et al. Adverse birth outcomes associated with maternal smoking and polymorphisms in the N-Nitrosamine-metabolizing enzyme genes NQO1 and CYP2E1. Am J Epidemiol. 2008;167(6):719–26.
31. Yila TA, Sasaki S, Miyashita C, et al. Effects of maternal 5,10-methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and tobacco smoking on infant birth weight in a Japanese population. J Epidemiol. 2012;22(2):91–102.
32. Braimoh TS, Kobayashi S, Sata F, et al. Association of prenatal passive smoking and metabolic gene polymorphisms with child growth from birth to 3 years of age in the Hokkaido Birth Cohort Study on environment and Children's health. Sci Total Environ. 2017;605–606:995–1002.
33. Kobayashi S, Sata F, Sasaki S, et al. Genetic association of aromatic hydrocarbon receptor (AHR) and cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1) polymorphisms with dioxin blood concentrations among pregnant Japanese women. Toxicol Lett. 2013;219(3):269–78.
34. Smith GD, Ebrahim S. What can Mendelian randomisation tell us about modifiable behavioural and environmental exposures? BMJ. 2005;330:1076–9.
35. Nafee T, Farrell W, Carroll W, Fryer A, Ismail K. Review article: epigenetic control of fetal gene expression. BJOG. 2008;115(2):158–68.
36. Hackett JA, Surani MA. DNA methylation dynamics during the mammalian life cycle. Philos Trans R Soc Lond Ser B Biol Sci. 2013;368(1609):20110328.
37. Kobayashi S, Azumi K, Goudarzi H, Araki A, Miyashita C, Kobayashi S. Effects of prenatal perfluoroalkyl acid exposure on cord blood IGF2/H19 methylation and ponderal index: the Hokkaido study. J Expo Sci Environ Epidemiol. 2017;27:251–9.

38. Leung Y-K, Ouyang B, Niu L, et al. Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a Faroese birth cohort. Epigenetics. 2018;13(3):290–300.
39. Yu X. Association of prenatal organochlorine pesticide-dichlorodiphenyltrichloroethane exposure with fetal genome-wide DNA methylation. Life Sci. 2018;200:6–86.
40. Kingsley SL, Kelsey KT, Butler R, et al. Maternal serum PFOA concentration and DNA methylation in cord blood: a pilot study. Environ Res. 2017;158:174–8.
41. Miura R, Araki A, Miyashita C, et al. An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoroalkyl substance exposure: the Hokkaido study. Environ Int. 2018;115:21–8.
42. Chen CH, Jiang SS, Chang IS, Wen HJ, Sun CW, Wang SL. Association between fetal exposure to phthalate endocrine disruptor and genome-wide DNA methylation at birth. Environ Res. 2018;162:261–70.
43. Solomon O, Yousefi P, Huen K, et al. Prenatal phthalate exposure and altered patterns of DNA methylation in cord blood. Environ Mol Mutagen. 2017;58(6):398–410.
44. Hogg K, Price EM, Hanna CW, Robinson WP. Prenatal and perinatal environmental influences on the human fetal and placental epigenome. Clin Pharmacol Ther. 2012;92(6):716–26.
45. Ankley GT, Bennett RS, Erickson RJ, et al. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. Environ Toxicol Chem. 2010;29(3):730–41.
46. Kishi R, Zhang JJ, Ha EH, et al. Birth cohort consortium of Asia (BiCCA) – current and future perspectives. Epidemiology. 2017;28(1):S19–34.
47. Vrijheid M, Casas M, Bergstrom A, et al. European birth cohorts for environmental health research. Environ Health Perspect. 2012;120(1):29–37.
48. Early Growth Genetics Consortium. https://egg-consortium.org/. Accessed 1 July 2019.
49. Middeldorp CM, Felix JF, Mahajan A, et al. The early growth genetics (EGG) and EArly genetics and Lifecourse epidemiology (EAGLE) consortia: design, results and future prospects. Eur J Epidemiol. 2019;34(3):279–300.
50. Korevaar TIM, Taylor PN, Dayan CM, Peeters RP. An invitation to join the consortium on thyroid and pregnancy. Eur Thyroid J. 2016;5(4):277.
51. Kishi R, Araki A, Minatoya M, Itoh S, Goudarzi H, Miyashita C. Birth cohorts in Asia: the importance, advantages, and disadvantages of different-sized cohorts. Sci Total Environ. 2018;615:1143–54.
52. Barker D, Eriksson J, Forsen T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002;31:1235–9.
53. Gluckman P, Hanson M, Cooper C, Thornburg K. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 2008;359:61–73.
54. Newman J, Ross M. Early life origins of human health and disease. Basel: Karger; 2009.
55. Haugen AC, Schug TT, Collman G, Heindel JJ. Evolution of DOHaD: the impact of environmental health sciences. J Dev Orig Health Dis. 2015;6(2):55–64.
56. Environmentally-induced developmental origins of health and disease. https://cordis.europa.eu/project/rcn/106798/factsheet/en. Accessed 1 July 2019.
57. Hanson MA, Skinner MK. Developmental origins of epigenetic transgenerational inheritance. Environ Epigenet. 2016;2(1):dvw002.
58. Fleming TP, Watkins AJ, Velazquez MA, et al. Origins of lifetime health around the time of conception: causes and consequences. Lancet. 2018;391(10132):1842–52.
59. Himeno S, Aoshim K, editors. Cadmium toxicity, new aspects in human disease, rice contamination, and cytotoxicity. Singapore: Springer; 2019.
60. Stockhorm convention. http://www.pops.int/. Accessed 1 July 2019.
61. Ae R, Nakamura Y, Tada H, et al. An 18-year follow-up survey of dioxin levels in human milk in Japan. J Epidemiol. 2018;28(6):300–6.
62. Ingelido AM, Abate V, Abballe A, et al. Concentrations of polychlorinated dibenzodioxins, polychlorodibenzofurans, and polychlorobiphenyls in women of reproductive age in Italy: a human biomonitoring study. Int J Hyg Environ Health. 2017;220(2, Part B):378–86.

63. van den Berg M, Kypke K, Kotz A, et al. WHO/UNEP global surveys of PCDDs, PCDFs, PCBs and DDTs in human milk and benefit–risk evaluation of breastfeeding. Arch Toxicol. 2017;91(1):83–96.
64. Tsai M-S, Miyashita C, Araki A, et al. Determinants and temporal trends of perfluoroalkyl substances in pregnant women: the Hokkaido study on environment and Children's health. Int J Environ Res Public Health. 2018;15(5):989.
65. Ministry of Health Labour, and Welfare, Japan. Booklet of occupational hygiene (in Japanese).
66. Sajiki J, Yonekubo J. Leaching of bisphenol A (BPA) from polycarbonate plastic to water containing amino acids and its degradation by radical oxygen species. Chemosphere. 2004;55(6):861–7.
67. The European Commission. Commission Regulation (EU) 2018/2005 of 17 December 2018.
68. Okada E, Kashino I, Matsuura H, et al. Temporal trends of perfluoroalkyl acids in plasma samples of pregnant women in Hokkaido, Japan, 2003–2011. Environ Int. 2013;60:89–96.
69. Wang M, Park JS, Petreas M. Temporal changes in the levels of perfluorinated compounds in California women's serum over the past 50 years. Environ Sci Technol. 2011;45(17):7510–6.
70. Glynn A, Berger U, Bignert A, et al. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996–2010. Environ Sci Technol. 2012;46(16):9071–9.
71. Gyllenhammar I, Glynn A, Jönsson BAG, et al. Diverging temporal trends of human exposure to bisphenols and plastizisers, such as phthalates, caused by substitution of legacy EDCs? Environ Res. 2017;153:48–54.
72. Zota AR, Calafat AM, Woodruff TJ. Temporal trends in phthalate exposures: findings from the national health and nutrition examination survey, 2001–2010. Environ Health Perspect. 2014;122(3):235–41.
73. Noel-Brune M, Goldizen FC, Neira M, et al. Health effects of exposure to e-waste. Lancet Glob Health. 2013;1(2):e70.
74. Grandjean P, Abdennebi-Najar L, Barouki R, et al. Timescales of developmental toxicity impacting on research and needs for intervention. Basic Clin Pharmacol Toxicol. 2018;00:1–11.
75. WHO and UNEP. Global assessment of the state-of-the-science of endocrine disruptors. 2002.
76. WHO and UNEP. State of the science of endocrine disrupting chemicals—an assessment of the state of the science of endocrine disruptors prepared by a group of experts for the United Nations Environment Programme (UNEP) and WHO. 2012.
77. Shape the Future of Life: Healthy Environments for Children. https://www.who.int/world-health-day/previous/2003/press/announcement2/en/. Accessed 1 July 2019.