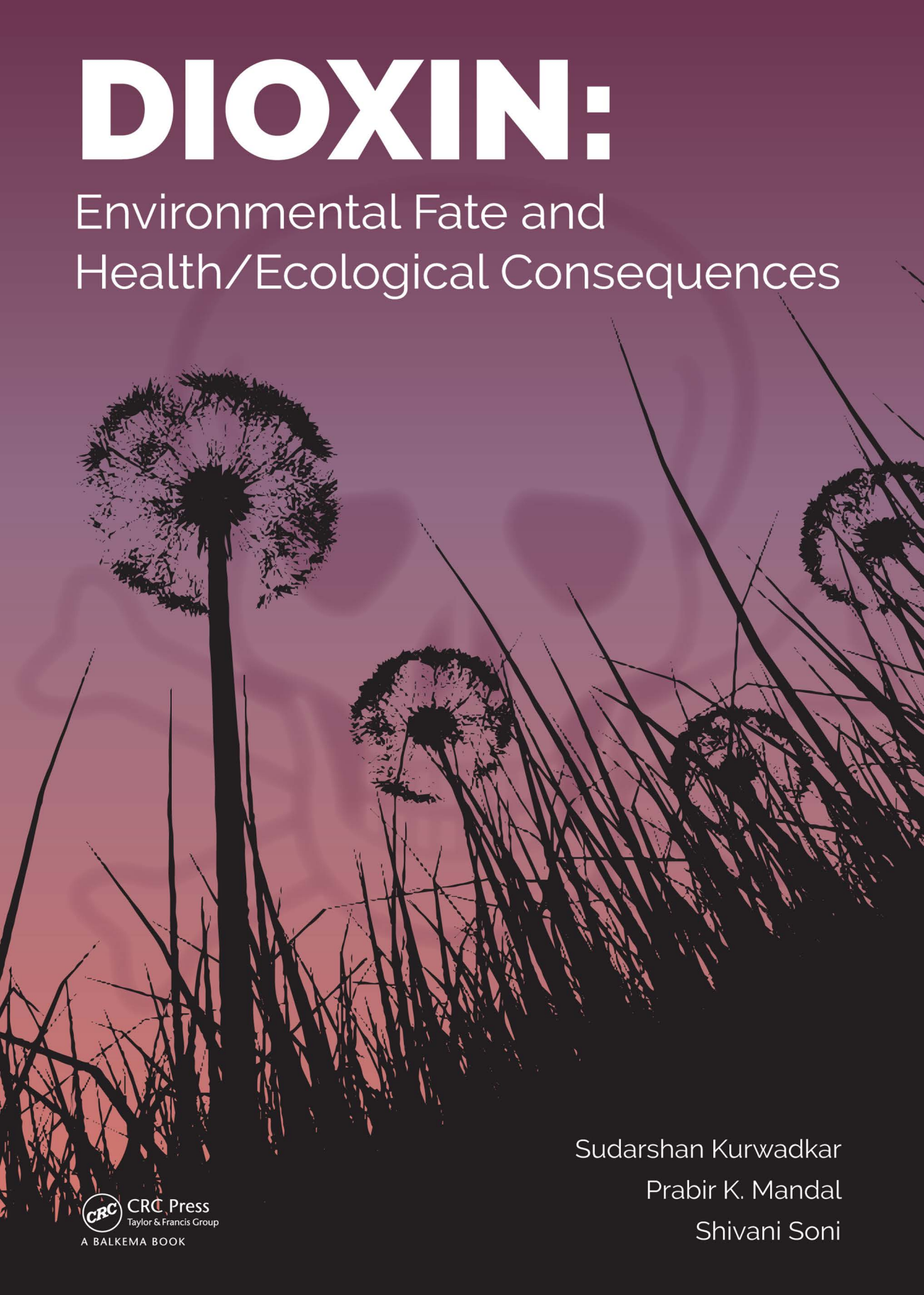
# DIOXIN:

## Environmental Fate and Health/Ecological Consequences



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CRC Press
Taylor & Francis Group
A BALKEMA BOOK

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CRC Press
Taylor & Francis Group
Boca Raton London New York Leiden

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

A BALKEMA BOOK

*CRC Press/Balkema is an imprint of the Taylor & Francis Group, an informa business*



Typeset by Apex CoVantage, LLC



Library of Congress Cataloging-in-Publication data
*Applied for*

Published by: CRC Press/Balkema
Schipholweg 107C, 2316 XC Leiden, The Netherlands
e-mail: Pub.NL@taylorandfrancis.com
www.crcpress.com – www.taylorandfrancis.com

ISBN: 978-1-138-04724-2 (Hbk)
ISBN: 978-1-315-17096-1 (eBook)
DOI: https://doi.org/10.1201/9781315170961

# Contents

# Preface

The occurrence of a variety of hazardous pollutants in environmental matrices is routinely documented in the scientific journals. Often, such information remains confined to journals, unless exposure to hazardous pollutants, and subsequent impacts on human health and the environment, gain widespread public attention. While working as an Environmental Engineer with the Missouri Department of Natural Resources – Division of Environmental Quality, I learned about the widespread contamination and subsequent evacuation of the dioxin-contaminated city of Times Beach (aka present-day Route 66 State Park), St Louis County, Missouri. The magnitude of dioxin contamination and human health exposure generated renewed focus on managing the pollution from persistent organic pollutants such as dioxin. Now, more than three decades later, exposure to dioxin and its impact on human health is still a concern. The high persistence of dioxin in environmental matrices means that they are stable towards degradation, have high bioaccumulation potential, and likely to enter the food web. Though the use of dioxin is significantly reduced, continued exposure to dioxin is being reported primarily from past dioxin contamination.

Dioxins represent a family of highly toxic and persistent organic pollutants that have severe human health implications. Dioxin and dioxin-like compounds are not intentionally produced; instead, they are the by-products of industrial processes, such as the production of specific organic chemicals, metals smelting, and refining. Furthermore, combustion of organic compounds, such as municipal waste, hazardous waste, medical waste, and compounds containing chlorophenols also produces dioxins. According to the U.S. Environmental Protection Agency (USEPA), even the backyard burning of waste material is known to produce higher levels of dioxins compared to the controlled combustion in a regulated facility. According to one estimate, there are 210 possible congeners in the dioxin family that includes 75 polychlorinated dibenzo-p-dioxin and 135 polychlorinated dibenzofurans with each form having different physical and chemical properties. Historically, even though the total environmental release of dioxins is on the decline due to stringent regulations, the routes and exposure to dioxin and its toxicity is still a cause for concern.

The toxicity associated with dioxin, its persistence, and the magnitude of its usage is almost at par with that of the legacy chemicals like DDT. Dioxin and dioxin-like compounds are so toxic that there is no baseline or threshold below which it will not cause cancer. It is this fact that is most concerning. Human exposure to dioxin or dioxin-like compounds occurs through various routes and pathways. Ingestion of dioxin-contaminated fish, meat, and dairy products is one of the significant exposure pathways. Over-dependence on these products will lead to widespread exposure to these compounds. According to one estimate, adults in the United States receive 42% of the toxic equivalent of dioxin through the

dietary intake of freshwater fish, beef, and dairy products. Given the magnitude of the exposure and toxicity of the dioxin, it is imperative that readers should know the state of scientific know-how on dioxin and dioxin-like compounds.

The book provides the reader with a holistic understanding of the most notoriously toxic compound dioxin. It will give the reader an in-depth toxicological profile about human health consequences, and it also will walk the reader through various routes and exposure pathways of dioxin and dioxin-like compounds. The book is designed in three sections. The first section documents the occurrence of dioxin in various matrices, such as air, water, soil, and food while the second section deals with the toxicity of dioxin and its implication to human health and ecology. The last section of the book deals with the remediation techniques for removal of dioxin from the aqueous and terrestrial environment. Dioxin is a persistent organic pollutant, its occurrence and exposure are almost imminent, requiring its removal from the environment. We hope you will find the sequential arrangement of book chapters enjoyable to read. We designed this book in a way that will primarily address the needs of a variety of disciplines, including chemistry, biochemistry, toxicology, environmental science, environmental health, and environmental engineers. We enjoyed putting this book together and hope that you will find it helpful.

*Sudarshan Kurwadkar*
*Prabir Mandal*
*Shivani Soni*

# About the editors

**Dr. Sudarshan Kurwadkar** is an Associate Professor in the Civil and Environmental Engineering Department at California State University, Fullerton. Before starting his academic career, he worked as an Environmental Engineer with the Missouri Department of Natural Resources' Division of Environmental Quality. His research interests are in the broadly defined area of fate and transport of emerging contaminants in the aquatic and terrestrial environment. His ongoing research involves the occurrence and persistence of neonicotinoid insecticides, which is the hotly debated topic in the world. He is a Board Certified Environmental Engineer and a licensed Professional Engineer in many states. He is a recipient of several awards, scholarships and fellowships including, Teaching Excellence Award; Outstanding Faculty Advisor Award (Los Angeles Section); Outstanding Faculty Advisor Award (Orange County Section); Excellence in Scholarly and Creative Activities Award; Chi-Epsilon Scholarship for academic excellence; ASEE Early Career Award; DOE Visiting Faculty Fellowship; Air Force Institute of Technology Summer Faculty Fellowship; John and Susan Mathes Doctoral Fellowship; and Global Initiative of Academic Networks Fellowship. Dr. Kurwadkar is an ASCE Vice President for student affairs and serves on the Board of Directors of the Los Angeles ASCE Section. He is popularly called 'Dr. K' and highly regarded by students as a fantastic advisor who flawlessly mixes humor and academic rigor and makes learning a pleasant experience. His dedication to student success, personalized attention to student design teams, and student rapport is simply unparalleled. His commitment to student success is widely acknowledged. Besides teaching and research, he enjoys outdoor activities such as swimming, kayaking, biking, fishing, and long-distance traveling. He has driven cross-country from Los Angeles, CA to Kittery, ME, visiting major tourist attractions all along.

**Dr. Prabir K. Mandal**, Ph.D. (Genetics) is a full Professor of Biology and Chair of the Department of Mathematics & Sciences at Edward Waters College, Jacksonville, FL. He has been awarded the Distinguished Professor of the College during 2012-2013 and President's Ideal Faculty in 2010 for excellence in teaching, research, scholarship, service, adhering to policy and for taking the initiative to advance the college through program development. He also serves as a member of the steering committee for the Health Equity Research Institute and is a member of the Health Disparities Research Advisory Committee, Florida. Dr. Mandal is involved in curriculum reform, mentoring, and spearheading new initiatives for Allied Health education, focusing on the underserved minority population, specifically African-Americans in the State of Florida.

**Dr. Shivani Soni** joined as a lecturer at Chapman University and California State University, Fullerton, in 2016. She worked as an associate professor in the Department of Biological Sciences at Alabama State University till July 2016. She completed her Ph.D. in Molecular Parasitology in 2004 from University of Delhi, India. She did her postdoctoral research at Tufts School of Medicine, Boston, Massachusetts. Later she joined as a senior postdoctoral fellow at Health Science & Technology division of Harvard Medical School-MIT, Boston, Massachusetts. Her research interests are in the area of Hematology and Oncology. Her research has focused on the field of erythropoiesis (Red blood cell formation), where she demonstrated the role of a novel protein Emp (Erythroblast Macrophage Protein) in red blood cells and macrophage development. Emp was discovered by Dr. Hanspal in 1994, and Dr. Soni was the first one to characterize its function in vivo in 2006. This significant discovery has resulted in multiple high impact publications and a prestigious travel award from American Society of Hematology. Her current research work is to elucidate the role of Emp in colorectal cancer progression and to explore its value as predictive & prognostic biomarkers. In collaboration, she has developed several novel polymeric hybrid Nano formulations for cancer chemotherapy and targeting aberrant signal transduction pathways, which have led to many publications in peer-reviewed journals as well as patents.

# Occurrence, fate, and distribution of dioxin in the environment

Chapter 1

# Dioxin

## History, environmental occurrence, and human health and ecological consequences

*Sudarshan Kurwadkar*

Dioxin is a general term applied to a group of compounds that are the unintentional by-product of anthropogenic activities such as incineration, combustion, industrial and manufacturing operations. These compounds are a class of structurally related halogenated aromatic hydrocarbons, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) or polychlorinated dibenzo-p-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF) (Figure 1.1). One of the most common and also one of the most toxic forms of dioxin is 2,3,7,8-TCDD, which is formed during the manufacturing of 2,4,5-trichlorophenol (2,4,5-TCP). Chemical characteristics of dioxins include their low water solubility, low vapor pressure, high melting point, and are lipophilic, hydrophobic, and bioaccumulative (Srogi, 2008; Boalt et al., 2013). These properties make dioxins persistent organic pollutants POPs. Coupled with their long-range atmospheric transport (LRAT), they are ubiquitously

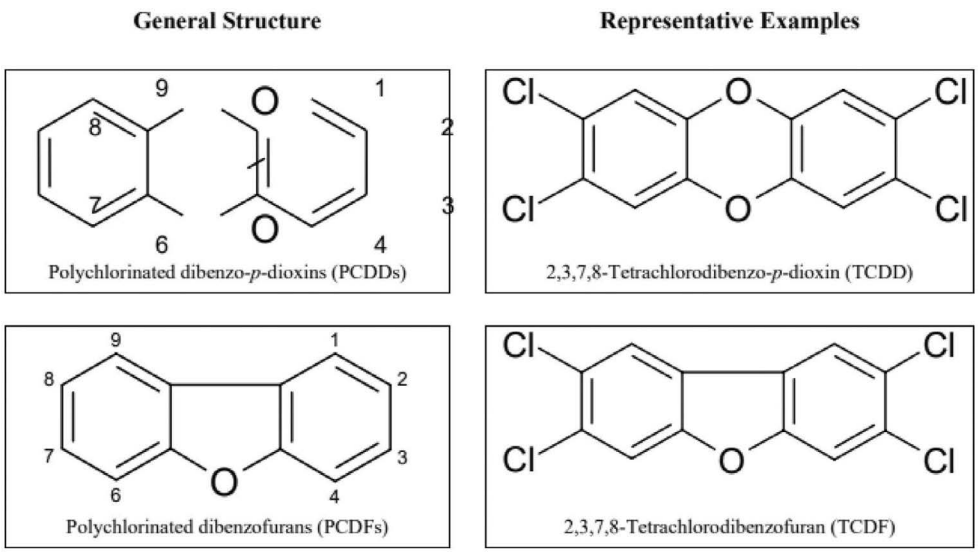


*Figure 1.1* General molecular structure of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) and their respective representative examples.

(Source: USEPA, 2008)

found in all environmental matrices. Of the 75 congeners of dioxins, 2,3,7,8-TCDD has been extensively investigated due to its acute toxicity. Because of its toxicity, dioxins are included in the United Nations Environment Program's (UNEP) list of "Dirty Dozen," a list of persistent organic pollutants proposed to be eliminated through various global treaties. Although historical releases of dioxin have been primarily attributed to the chemical industry, other uses of chloro-organic compounds are also substantial. This chapter discusses the chronology of historical milestones that bought dioxin as a mainstream pollutant, the magnitude of pollution, current sources, toxicity, and the exposure routes and resulting human health impact. The chapter also provides a summary of the evolution of regulatory response to mitigate the risk due to exposure to dioxin and provides an insight into the current trends and status of dioxin pollution.

## 1 Dioxin – historical perspective

Formation of dioxin-like compounds was first reported in the late 19th century. In 1929, Monsanto started the production of polychlorinated biphenyls (PCB) in Anniston, Alabama, and dioxin-like substances were the resultant by-product of the manufacturing of PCB (Commoner *et al*., 1994; Earthjustice, 2019). Large scale pollution of organochlorine-based substances during the early 20th century has led to the unintentional production of dioxin and dioxin-like compounds. The earliest recognized instance (documented in 2007) of high levels of dioxin production resulting from industrial activities dates back to 1900s (Weber *et al*., 2008). The authors reported that a German Leblanc Soda factory that operated from 1848–1893 produced 1 to 10 kg toxic equivalent (TEQ) of dioxin (PCDFs). Note that, human beings are likely to be exposed to the mixtures of dioxins, furans, and other congeners through a variety of exposure routes and pathways, and as such accurate risk quantitation is difficult. Toxic Equivalent (TEQ) or Toxic Equivalent Factors (TEF) are used to show the relative toxicity of the compound compared to its most toxic member. These factors are essentially the relative toxicity of dioxin compounds compared to its most toxic form typically 2,3,7,8-TCDD (USEPA, 2007).

Another dioxin compound has become well known through a series of events that made dioxin a household name. With the use of "Agent Orange" during the Vietnam War, it achieved worldwide notoriety because of its long-term human health impact due to exposure to dioxin. During 1960 to 1972, The Syntex Facility in Verona, MO was operated by Hoffman-Taff, Inc. In the early 1960s, this facility was located on 180 acres and produced 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T). In 1968, the company leased out part of the facility's building to Northeastern Pharmaceutical and Chemical Company (NEPACCO) to produce the disinfectant hexachlorophene. Both the production of 2,4,5-T and hexachlorophene led to the unintentional production of 2,3,7,8-TCDD leading to the contamination of soil, groundwater, and the aquatic biota downstream in the Spring River with dioxin and other volatile organic compounds (MDNR, 2019). According to one estimate, approximately 12,000 gallons of concentrated 2,3,7,8-TCDD filter cakes were incinerated; 4,300 gallons stored onsite; and 18,000 gallons were transported (Eastern Missouri) and subsequently sprayed on the dirt roads, riding arenas, and truck lots. An additional 25,000 gallons of 2,3,7,8-TCDD was sent to Waste Management School in Neosho, MO (Powell, 1984).

Pollution due to dioxin and dioxin-like substances received widespread public attention due to the human health consequences arising from its indiscriminate use during the Vietnam War and also due to other human-made disasters, such as the Times Beach incident, Love Canal, and the industrial accident in Seveso, Italy. For example, during 1965–1971, extensive use of "*Agent Orange*" a defoliating phenoxy herbicide comprising a 50/50 mixture of 2,4-dichlorophenoxyacetic acid (2,4-D) and acid (2,4,5-T) led to the contamination of 2,3,7,8-TCDD. Nobody can accurately determine how much dioxin mass was actually applied – according to some estimates, the total mass of 150–680 kg of 2,3,7,8-TCDD could have been added to the environmental system of Southern Vietnam (Dwernychuk *et al*., 2002; Hites, 2011). Another incidence of dioxin pollution that received widespread press coverage was the magnitude of dioxin contamination at Times Beach, a site located within the flood plain of the Meramec River in Eastern Missouri. During 1972–1976, the unpaved dirt roads at this site were sprayed with waste oil containing 2,3,7,8-TCDD as a dust suppressant (Powell, 1984; Hites, 2011). Analysis of more than 5200 samples analyzed so far revealed that the highest 2,3,7,8-TCDD concentration at this site was 350 ppb, 2.2% samples with concentration greater than 100 ppb, while 22% of the has concentrations greater than 1 ppb. A similar instance of dioxin pollution was also reported at Love Canal, Niagara Falls, NY with the highest recorded concentration of 2,3,7,8-TCDD in sediments was reported as 312 ppb (Rappe, 1984). An incidence of dioxin pollution from industrial activity was also reported from Seveso, a town in Northern Italy. In 1976, an explosion at the chemical plant led to the airborne discharge of dioxin, and subsequent soil deposition resulted in massive soil contamination with concentrations in some parts higher than 50 $\mu g/m^2$ (Hites, 2011). According to one estimate, more than 37,500 people in the immediate vicinity could have been exposed to dioxin (Pope and Rall, 1995). Some of these instances are now recorded among the most notable environmental disasters of the 20$^{th}$ century.

## 2 Sources of dioxin in the environment

Two primary sources through which dioxin enters the environment are natural sources and anthropogenic sources. Dioxin naturally exists in the environment, and some natural processes such as forest fires can also contribute to dioxin in the environment. Occasionally, uncontrolled forest fires, brush fires, landfill fires, accidental fires, building fires, and open burning of waste contribute to the emission of dioxin (USEPA, 2006). Natural sources of dioxin are few, and net mass released to the environment is relatively small compared to the dioxin releases from human activities. Dioxin is not an individual compound released directly from a source, but rather a group of compounds that are formed during the commercial production of chlorinated organic solvents, such as pesticides and herbicides, the incineration of municipal waste, and the combustion of fuels like wood, coal, diesel, or oil (Kanan and Samara, 2018) (Figure 1.2). They are also produced during a variety of industrial processes like pulp and paper production, chemical manufacturing, and metal processing (Mukherjee *et al*., 2016). Combustion is a broad term and includes combustion of municipal, solid waste, sewage sludge, medical waste, and hazardous waste (USEPA, 2006). In the past, chemical industries were the primary sources of dioxin in the environment. Other contemporary processes, such as metal smelting, (iron, lead, copper smelting) and steel, magnesium, and titanium dioxide production also contribute to dioxin in the

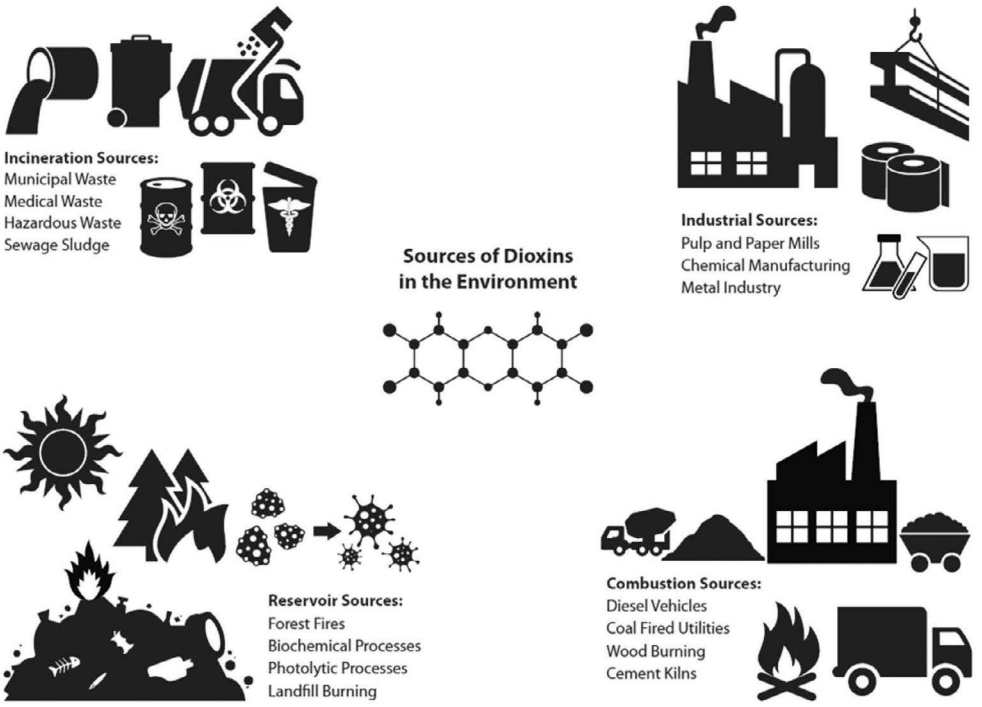


*Figure 1.2* Various natural and anthropogenic activities through which dioxin and dioxin-like compounds are emitted to the environment.

environment (USEPA, 2006). Primarily emitted from a variety of anthropogenic activities, dioxins are toxic, persistent, and bioaccumulative (Liu *et al*., 2013). In 2012, emissions of dioxins from electricity and heat generation processes (combustion of gasoline, diesel, wood, coal, and other fuel oils) accounted for the largest emission (66.2%) from all the controlled sources; while dioxins emissions from waste to energy, waste incineration, and metallurgical processes accounted for 0.5%, 1.9%, and 24% respectively (Dwyer and Themelis, 2015).

A variety of industrial processes, such as pesticide and herbicide production, result in the formation of 2,3,7,8-TCDD. For example, dioxins emissions from 2,4,5-TCP an essential chemical used in the manufacture of several pesticide products, including the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) releases dioxin as a byproduct. Although the PCDDs/Fs release to the environment peaked from 1939 to 1972, lately dioxin concentrations in the atmosphere have decreased significantly owing to the control technologies used in municipal waste combustors, chemical manufacturing, and industrial processes. According to one estimate, since 1987, dioxin emissions from various industrial sources in the US have decreased over 95% except for emissions from open burning processes that have increased by 43%. Frequent forest fires and large-scale consolidated landfill operations account for 77% of the open burning emissions and account for 93% of the net increase (Dwyer and Themelis, 2015). For better understanding about the release of dioxin and

dioxin-like substances in the environment, the USEPA categorized the emissions sources in six categories that include: contemporary formation sources and reservoir sources with and without a quantified release, and reservoir sources with preliminary release estimates. Although concerted efforts are being made to reduce the potential sources of dioxin contamination, the lingering effect of past contamination will remain for the foreseeable future.

## 3 Magnitude of dioxin pollution

While dioxin and dioxin-like compounds are one of the primary products of combustion/incineration, a theory was postulated that these compounds were in the environment since the "*advent of fire*" (Hites, 2011). Similar observations were also postulated by Bumb *et al*. (1980) through their "*Trace Chemistries of Fire*" theory. These studies supported their hypothesis by analyzing sediment deposits that showed concentrations at or below the method detection limits. Since the early 20th century is considered to be prechlororganic, it brings credence to these theories and hence the presence of PCDD/Fs in preindustrial society could be attributed to other sources, such as metal production and burning of fuels other than chlorine Kjeller and Rappe (1995). While other studies stated that concentrations of dioxins and furans in the early 1900s were low, they gradually entered the environment in the 1930s and peaked up during 1920s–1970s (Bhavsar *et al*., 2008). The extent of dioxin pollution is dependent on past pollution sources, geographical location (downwind from emission sources), and atmospheric transport and deposition. Due to these factors, it is difficult to accurately estimate the concentration of dioxin in the atmosphere originating from a particular source. Historical trends in dioxin concentrations in the atmosphere continued to increase from the early 1930s due to the advent of chemical industry (particularly the chlorine industry). In the United States, as of 1989, the total annual emissions of PCDD/Fs in the atmosphere from all known sources was approximately 400 kg, attributed exclusively to the combustion sources (Thomas and Spiro, 1995). Between 1987–2012, the majority of the sources showed continuous decline in dioxin emissions (Figure 1.3); however, emissions from uncontrolled open burning continues to increase and may threaten human and ecological health (Dwyer and Themelis, 2015).

The declining trend in dioxins in the atmosphere is consistent with the fact that production of PCBs ceased in the United States in 1979 (USEPA, 2015). According to the USEPA, between the years1987–2000, a nearly 90% reduction in emissions of dioxin and dioxin-like substances from all known sources was achieved. This extensive reduction in emissions of these compounds was primarily due to controlling emissions from primary sources, such as combustion of municipal waste (USEPA, 2006).

## 4 Global distribution of dioxin

Once dioxins are released to the atmosphere or land, LRAT of dioxin will lead to their occurrence farther from the source. Hypothetically speaking, a country need not have to be an active emitter of dioxins, but its proximity to the active emission sources will determine the amount of dioxin it will receive. Interestingly, environmental factors such as the hydrological cycle can further facilitate transport through the evaporation cycle in what is referred to as a "*grasshopper effect*" (Gouin *et al*., 2004). Furthermore, the global distribution of dioxin is not only impacted by the level of industrialization but also the distribution

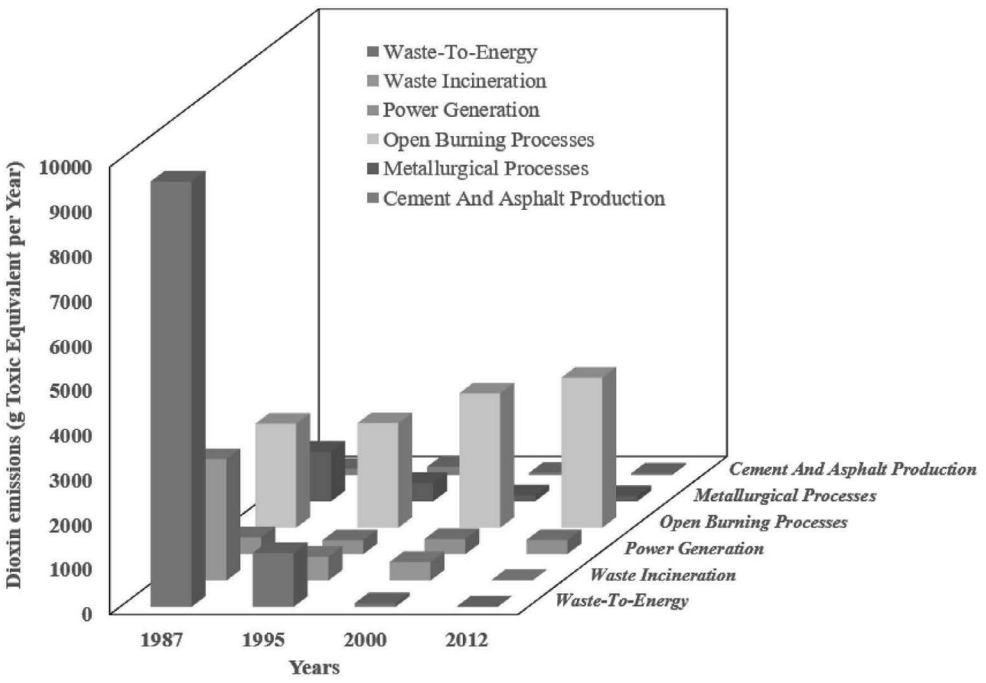


*Figure 1.3* Inventory of dioxin emissions from various sources.
Data Source: (Dwyer and Themelis, 2015)

to LRAT of dioxin. For example, dioxin emissions from industrial countries with higher economic activities (typically G-20 countries) account for more than 80% of the annual estimated emissions. In undeveloped countries, dioxin emissions are attributed to residential heating and cooking using traditional fuel sources and waste combustion (Booth *et al*., 2013). A study reported that the concentration of PCDD/F in Africa is at comparable levels to the concentration of PCDD/Fs found in the developed countries. The authors attributed elevated PCDD/Fs levels in Africa to industrial emissions, stockpiles of outdated pesticides, household heating, leakage from electricity generation plants and transformers, recycling of electronic waste, and incineration/combustion of household waste (Ssebugere *et al*., 2019). Given the complexity of global emissions, LRAT, and environmental factors, it is challenging to quantify the global distribution of dioxins accurately. Some studies have stated that the dioxin depositions were higher than the emissions and attributed this observation to the photochemical transformation of wood preservatives to dioxin (Booth *et al*., 2013). A similar mass imbalance between the emission of PCDD/F and their deposition is also reported by other authors (Wagrowski and Hites, 2000). According to the modeling estimate, overall annual global dioxin emission/production is estimated as 17,226 kg (287 kg TEQ), of which nearly 57% was deposited on soil, 40% on ocean water, and 3% remained in the atmosphere (Booth *et al*., 2013).

Stark differences in emission rates in different countries are also evidenced and can be attributed to the type of predominant activities emitting the dioxins. For example, PCDD/F contamination from the extensive use of pesticides in Japan during 1950–1998 is estimated

at 460 kg TEQ. While the wood treatment processes in Sweden has contributed to the release of 250 kg TEQ, these releases are substantial when compared to the recent releases of these compounds from nearly 55 countries which is estimated at 20 kg TEQ/yr. (Fiddler 2007; Weber *et al*., 2008). An accurate estimate of global emissions of PCDD/F is difficult due to the magnitude of the problem and the lack of an acceptable and reproducible method of sampling and analysis being available.

The countries that ratified the Stockholm Convention on Persistent Organic Pollutants were mandated to establish the inventory of the PCDD/F emissions. The UNEP has developed a methodology that can be used by both developed and developing countries to quantify the release of PCDD/F. Table 1.1 and 1.2 shows the atmospheric emissions of dioxin in 23 countries across five continents, including developed and developing countries representing a wide range of size, population, industrial, and economic development. The estimated mass of dioxins emissions from developed countries is in the range of 7000 and 13,000 g TEQ per year, while the median and total atmospheric emissions from developing countries is 12 μg TEQ/person and 21 μg TEQ/person (Table 1.1 and 1.2), which is remarkably low (Fiedler, 2007).

Mapping of oceanic atmosphere for the presence of PCDD and PCDF shows considerable variety in concentrations of these compounds. Detailed analysis of samples collected

*Table 1.1* Inventory of global annual atmospheric emissions of PCDD/F using the Toolkit application developed by the UNEP (Fiedler, 2007) (Reproduced with permission from Chemosphere, Elsevier)

| *Country/State* | *g TEQ/a* | | *Reference year* | *Reference* |
|---|---|---|---|---|
| | *Best* | *Max* | | |
| Australia | 150 | 2300 | 1998 | UNEP (1999) |
| Austria | 29 | | 1994 | UNEP (1999) |
| **Belgium** | 661 | | 1995 | UNEP (1999) |
| Switzerland | 181 | | early 1990s | UNEP (1999) |
| Canada | 164 | | 1999 | Environment Canada (2001) |
| Croatia | 95.5 | | ca. 1997 | UNEP (1999) |
| Czech Republic | 179 | | 2001 | RECETOX (2003) |
| Denmark | 19 | 170 | 1998/99 | COWI (2001) |
| Finland | 98.3 | 198 | ca. 1997 | UNEP (1999) |
| France | 380 | | 2002 | CITEPA (2004) |
| Germany | 323 | | 1994 | UNEP (1999) |
| Hong Kong SAR | 23 | 33 | 1997 | Hong Kong (2000) |
| Hungary | 103 | | 1998 | UNEP (2000) |
| Ireland | 34 | | 2000 | Hayes and Marnane (2000) |
| Japan | 372 | 400 | 2003 | MoE (2004) |
| The Netherlands | 486 | | 1991 | UNEP (1999) |
| New Zealand | 14 | 51 | 1998 | Buckland *et al.* (2000), Dyke *et al.* (2000) |
| Norway | 9.15 | | ca. 1997 | UNEP (1999) |
| Sweden | 22 | 88 | 1993 | UNEP (1999) |
| Slovak Republic | 616 | | 1996 | UNEP (2000) |
| Taiwan | 67.3 | | 2000 | Chen (2004) |
| United Kingdom | 560 | 1099 | | UNEP (1999) |
| United States of America | 2501 | 4901 | 1995 | US-EPA (2000) |
| Global flux | 7087 | 12570 | | |

*Table 1.2* Annual atmospheric emissions of PCDD/F per country to air and other sources using the Toolkit application developed by the UNEP (Fiedler, 2007) (Reproduced with permission from Chemosphere, Elsevier)

| *Country* | *Pop … mio.* | *Annual releases to g TEQ/a* | | *Annual releases μg TEQ/person. a* | |
|---|---|---|---|---|---|
| | | *Air* | *Total* | *Air* | *Total* |
| Argentina | 37.4 | 874 | 2111 | 23 | 56 |
| Australia | 19.7 | 495 | 1800 | 25 | 91 |
| Brunei | 0.34 | 0.75 | 1.40 | 2.2 | 4.1 |
| Cambodia | 13.4 | 273 | 607 | 20 | 45 |
| Chile | 15.7 | 51.9 | 85.6 | 3.3 | 5.5 |
| Croatia | 4.497 | 116 | 168 | 26 | 37 |
| Cuba | 11.2 | 195 | 319 | 17 | 28 |
| Ecuador | 13.7 | 65.5 | 98.5 | 4.8 | 7.2 |
| Estonia | 1.42 | 14 | 29.2 | 10 | 21 |
| Jordan | 5.3 | 64.3 | 81.6 | 12 | 15 |
| Latvia | 3.4 | 22.0 | 54.5 | 6.5 | 16 |
| Lebanon | 3.7 | 79.0 | 165.8 | 21 | 45 |
| Lithuania | 3.6 | 17 | 35.8 | 4.7 | 10 |
| Mauritius | 1.21 | 16.5 | 26.5 | 14 | 22 |
| Paraguay | 5.2 | 70.7 | 156 | 14 | 30 |
| Philippines | 84.5 | 328 | 534 | 3.9 | 6.3 |
| Poland | 38.6 | 490 | 1039 | 13 | 27 |
| Seychelles | 0.081 | 4.1 | 5.4 | 51 | 67 |
| Sri Lanka | 19.9 | 172 | 257 | 8.6 | 13 |
| Thailand | 62.4 | 286 | 1070 | 4.6 | 17 |
| Uruguay | 3.3 | 18.7 | 48.5 | 5.7 | 15 |
| Vietnam | 78.4 | 16.0 | 69 | 0.2 | 0.9 |
| Zambia | 10.3 | 290 | 483 | 28 | 47 |
| Total | | 3959 | 9445 | Median: 12 | Median: 21 |

from the gas and aerosol phase of the oceanic environment suggests low concentrations in the Pacific Oceans except for coastal areas of Southwest California, Mexico, and Central America (Figure 1.4). Compared to the concentration in the Pacific Oceans, the North Atlantic has higher concentrations of both PCDD/F (Morales *et al*., 2014). Other studies based on the depth profiles of PCDD/Fs in sediment cores collected from the offshore and coastal area of the Baltic Sea Region suggest that PCDD/Fs in this region is primarily due to the LRAT of atmospheric emissions (Assefa *et al*., 2014).

## 5 Toxicity of dioxin

Exposure to both PCDD/F produces a broader toxic effect on animals. Not all dioxins have the same toxicity; however, they demonstrate a common mechanism to exert a toxic effect. For example, the most common toxic response of PCDD/F is its ability to bind to aryl hydrocarbon (Ah) – a cytoplasmic receptor protein (Srogi, 2008; Kulkarni *et al*., 2008). Since exposure to the general population occurs through a mixture of dioxin compound, it can have broader biological implications, including disruption of normal hormone signaling pathways and reproductive and developmental defects (Kulkarni *et al*., 2008). For

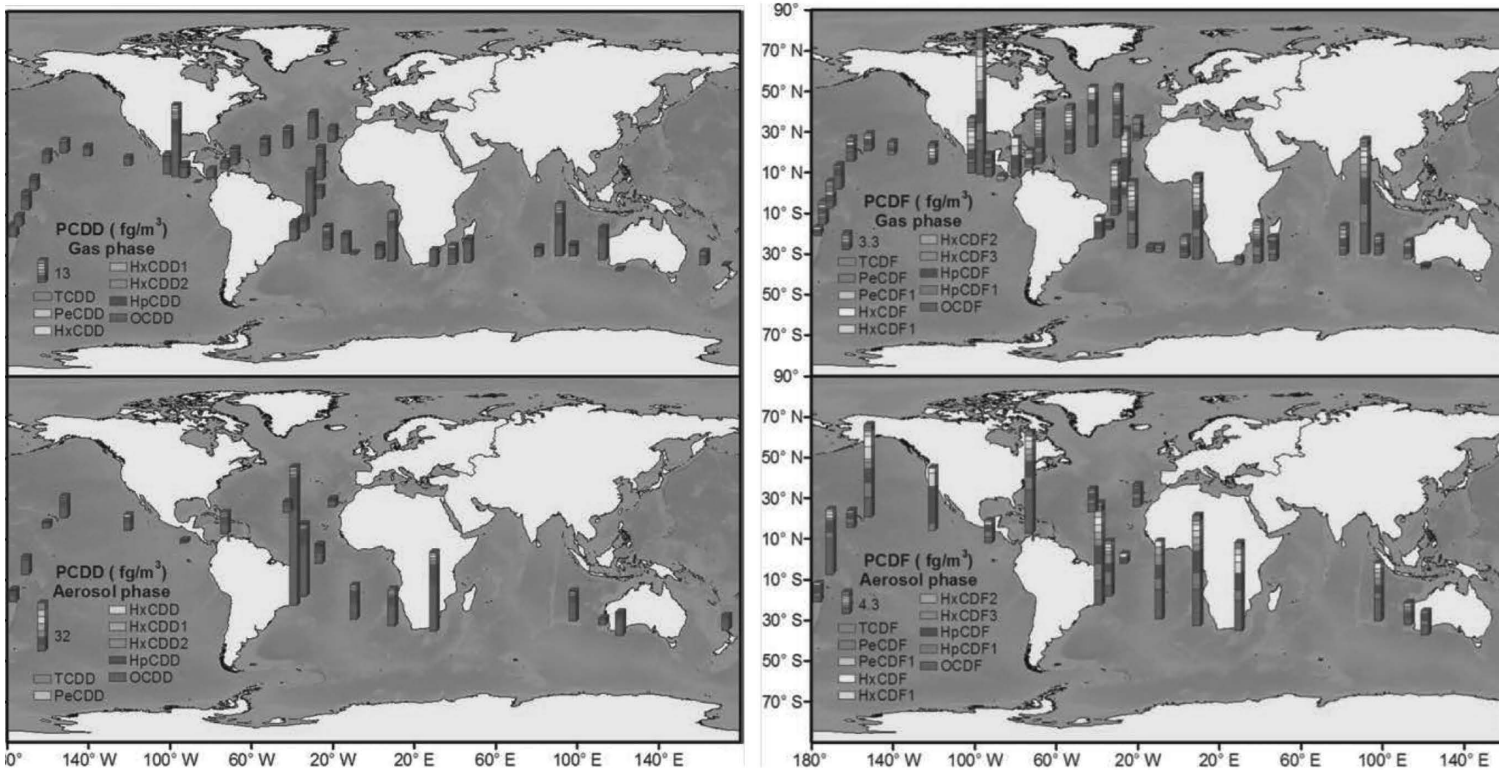


*Figure 1.4* Global distribution of PCDD (left) and PCDF (right) in the oceanic air in gas and aerosol phases.
(Source: Morales *et al.*, 2014. Reproduced with permission from American Chemical Society)

example, studies have shown that exposure to dioxin at levels ten times the level of current human exposure can decrease the sperm counts in rats and cause endometriosis in a rhesus monkey (Kogevinas, 2001). The toxicity of a particular dioxin compound is determined by the number of chlorine atoms it has and their configuration. For example, the dibenzo-p-dioxins have two benzene rings joined by two oxygen atoms and can have anywhere between zero to eight chlorine atoms attached to the benzene ring (Figure 1.1). Of the 75 congeners of PCDD and 135 congeners of PCDF, 17 congeners can have chlorine atoms placed in 2,3,7, and 8 of the parent molecule. These congeners can have different physical and chemical properties, which make them toxic, bioaccumulative, and resistant towards biotic and abiotic degradation. Based on the number of chlorine atoms and their configuration, a variety of congeners and of dioxin compounds can be formed with varying levels of toxicity (zero and one monochlorine dioxin being less toxic compared to dioxin with more than one chlorine atom) (Dopico and Gómez, 2015; Yang *et al*., 1999). Of the possible 210 congeners, 17 are toxic (Dopico and Gómez, 2015; Mukherjee *et al*., 2016) with 2,3,7,8-TCDD being the most toxic and often used as a reference to establish relative toxicity or the toxic equivalent of the rest of the dioxin congeners. Not surprisingly, the majority of the toxicity data is derived from high and low dosages of 2,3,7,8-TCDD. Another measure of evaluating toxicity is to establish the $LD_{50}$ values. These values indicate the lethal dose at which 50% of the exposed population will die. Various studies have experimentally demonstrated the $LD_{50}$ values for a variety of animal species exposed to 2,3,7,8-TCDD through oral administration. From Table 1.3, it can be seen that the $LD_{50}$ value varies considerably not only by species type but also by gender.

Often, the mass emissions data is expressed in terms of toxic equivalent, which is the weighted quantity of dioxin and dioxin-like compounds relative to the most toxic compounds in that category. It is a representation of the relative toxicity of dioxin compounds. It should be noted that the facility emitting a higher mass of low toxicity compounds may not necessarily be of public interest compared to a facility releasing a lower mass of highly toxic dioxin compounds (Watson, 2007). When evaluating the potential risk of dioxins, it is critical to account for their geographical mobility and toxic impact due to exposure from varied pathways.

*Table 1.3* Oral administration of 2,3,7,8-TCDD and determination of $LD_{50}$ values for various animal species (INSERM, 2000)

| *Species/strain (sex)* | *LD 50 (μg/kg)* |
|---|---|
| Guinea pigs Hartley (M) | 0.6–2.0 |
| Chickens NR | <25 |
| Monkeys rhesus (F) | 70 |
| Rats Sherman, Spartan | |
| (M) | 22 |
| (F) | 13–43 |
| Rats Sprague-Dawley (M) | 43 |
| Rats Fischer Harlan (M) | 340 |
| Mice C57BL/6 (M) | 181 |
| Mice DBA2/2D4 (M) | 2 570 |
| Mice B6D2F1 (M) | 296 |
| Rabbits/New Zealand | 115 |
| Syrian hamsters (M and F) | 1 157–5 051 |

*Note: M – Male, F – Female*

## 6 Routes and pathways of exposure to dioxin

Numerous sources through which dioxin enters the environment were already documented in the earlier section. Once released to the environment, the PCDDs and PCDFs are likely to be absorbed into particulate matter if not removed through photodegradation. Since they are less likely to volatilize, they tend to deposit on aquatic sediments (Kulkarni *et al*., 2008). Deposition of lipophilic dioxins on aquatic sediments can quickly bioaccumulate in the fatty tissues of animals and fish. Most plants (except zucchini and pumpkin) can uptake soil-deposited dioxin. Ingestion of such plants by grazing animals serves as one of the pathways through which dioxin enters the food web (Figure 1.5). Deposition of atmospheric dioxin on soil and plants and consumption of these contaminated plants is a primary pathway through which dioxin enters the animal food chain (Fries, 1995a). Not surprisingly, the food sources, animal-derived food, serve as a primary intake of dioxin in humans. Of all the exposure pathways, ingestion of food serves as a primary exposure route, while inhalation and dermal absorption pathways are not significant (Figure 1.5).

Besides exposure through the food chain, other exposure routes due to the ubiquitous presence of dioxin in various environmental matrices such as dioxin-contaminated air, water, and soil media are also possible. These dioxin exposure routes include inhalation, drinking water, dermal, and ingestion of food. Of all the potential exposure pathways, more than 90% of human exposure occurs through the oral pathway (ingestion of dioxin-contaminated food) through the food web, such as ingestion of dioxin-contaminated fish, vegetables, pork, poultry, and beef as well as dairy products (Figure 1.6) (Thrasher, 2014).

## 7 Exposure to dioxin and impact on biota

The incidence of chick edema disease in 1957 was the earliest documented adverse effect on biota, where several millions of young chickens were exposed to 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin through the chicken feed. Similar instances were also reported in the mid-1980s when the chickens were exposed to hepta- and octa-chlorinated dioxin and dibenzofuran congeners 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (Hites, 2011). Another incident of direct exposure to dioxin was the 1971 incident at Times Beach. Spent oil containing 300 ppm of 2,3,7,8-TCDD was sprayed indoors at Shenandoah stables indoor

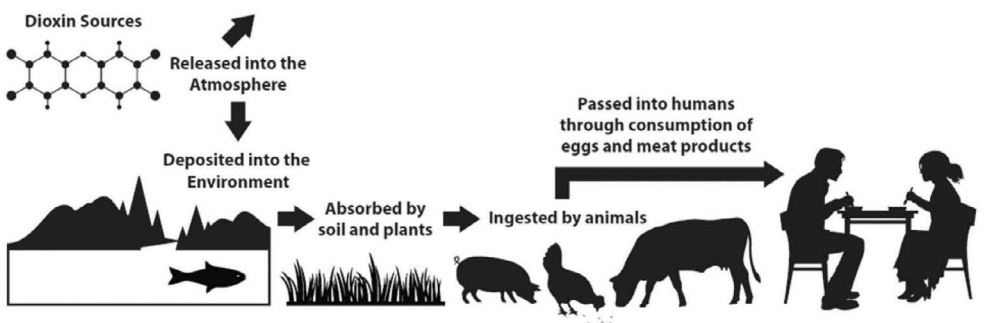


*Figure 1.5* Atmospheric pathways of dioxins' entry in the food web: emission → deposition → bioaccumulation/uptake → ingestion by animal → ingestion by humans.

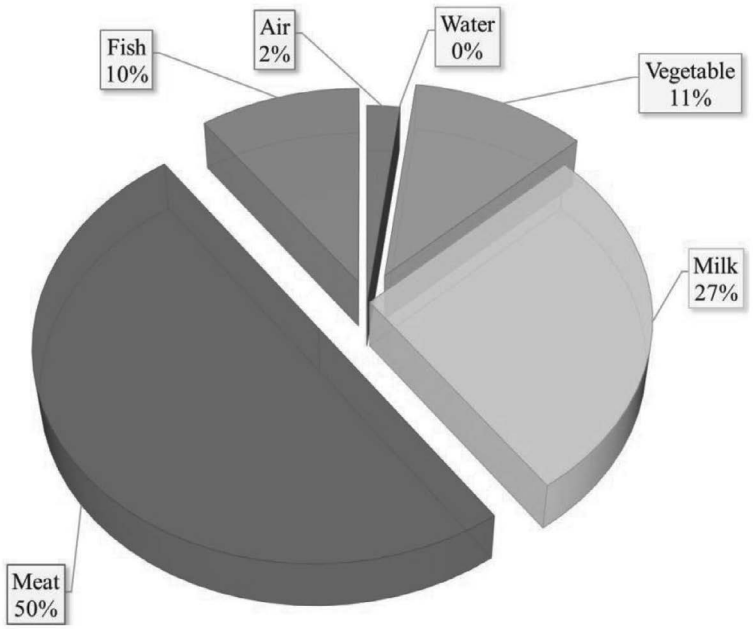


*Figure 1.6* Exposure of dioxin through various food sources. Intake is based on the average daily intake of dioxin.

(Source: Thrasher, 2014)

horse-riding arena, leading the death or euthanasia of 75 horses. In a week, small birds were also found dead in the arena (Hites, 2011).

## 8 Human health impact of exposure to dioxin

Persistence and widespread occurrence of dioxins in the environment (air, water, soil, plants, and animals) have increased the likelihood of human exposure to dioxin, which may have adverse implications to human health. As discussed earlier, a variety of exposure routes and pathways of dioxins are possible, and depending on the route, it may pose a varying risk to human health. The earliest known incident of toxicity due to exposure to dioxin was documented in the early 1930s when workers at Monsanto became sick (Commoner *et al*., 1994). Many of the widely discussed human health impacts due to exposure to dioxin originated with the July 10, 1976, Seveso incident at the ICMESA chemical plant located in a nearby town of Meda, on the northern outskirts of Seveso. A toxic cloud of dioxin (2,3,7,8-TCDD) was accidentally released through the chimney, exposing nearly 170 workers. According to some estimates, 30 kg TEQ of 2,3,7,8-TCDD was released, exposing people and contaminating the environment. Upon exposure, some people experienced skin conditions, the onset of chloracne – acne-like skin lesions or bumps and cysts on cheeks and behind the ears. Upon exposure to dioxin skewed sex ratio was observed in the exposed population of Seveso with more girls than boys being born to the exposed population (INSERM, 2000; Mocarelli, 2001).

In Europe, historical trends indicated increased exposure to dioxin with maximum exposure around 1950s–1960s and showed a gradual decline after that, with sudden spikes in dioxin exposure levels during the 1980s-1990s due to increased paper and pulp production and poorly controlled waste incineration (Kogevinas, 2001). Over the years, understanding of the human health impact due to exposure to dioxins has evolved. Exposure to dioxin can have a broad impact on human health, including gene expression, endocrine disruption, reproductive health, cancer (lymphomas, sarcomas, stomach cancer), skin lesions (chloracne), and immunological, hormonal, and developmental defects (Figure 1.7) (Kulkarni *et al*., 2008; Landi *et al*., 1997; Mitrou *et al*., 2001).

Increased incidence of cancer has been reported among industrial workers exposed to dioxin. However, the onset of cancer was not immediate. For example, people at the Seveso incident exposed to high levels of 2,3,7,8-TCDD have shown a slight increase in the incidence of cancer after five years. Studies also show that exposure to dioxin increases the risk of chloracne, Hodgkin's disease, non-Hodgkin's lymphoma, and soft tissue sarcoma (UNEP, 1999; Deriziotis, 2004). Inconsistencies in the incidence of cancer were reported, with some studies showing increased risk for some cancers (INSERM, 2000). Studies also reported contradictory findings on impairment of reproduction-related hormone levels. Additional associations between exposure to dioxin and altered liver function, lipid metabolism, and reduced immune system have also been reported (UNEP, 1999).

## 9 Evolution of regulatory perspective

Regulatory response to prevent adverse effects on human health appears to follow a similar trend with the continued increase in reported instances of exposure and resulting health consequences. Since dioxins were not intentionally produced, the regulations governing their release to the environment and consequently, human health or ecological impact, did not receive much impetus. Many reasons can be attributed to the lack of regulatory provisions or concern thereof due to little understanding of the risk involved due to exposure to dioxin. Even though the formation of 2,3,7,8-TCDD was recognized as early as 1974, nothing was done until a decade later when the Missouri Governor's task force declared that the whole basis of "half-life" applied to the persistence of dioxin in soil was inaccurate (Powell, 1984). Instances such as Times Beach and Love Canal have accelerated the pace of regulatory development. Table 1.4 shows the timeline of the evolution of regulatory response to managing dioxin pollution. The regulations evolved with the increased frequency of reported exposure and potential human health impact.

Some of the regulatory provisions that directly apply to dioxin include the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and Resource Conservation and Recovery Act (RCRA). The CERCLA (aka Superfund) was enacted by the U.S. Congress on December 11, 1980. This law includes provision to establish the liability of persons responsible for pollution, provide funds to remediate such site if the responsible party is not found, and directly respond to the release or perceived release of hazardous substances that may adversely affect human health or the environment. The Superfund was enacted in direct response to the discovery of massive human-made toxic dumps at Times Beach and Love Canal in early 1970s that required the responsible parties to take clean-up activities and to compensate for the cost of clean-up (Weber *et al*., 2008). Through CERCLA, the EPA has developed preliminary remediation goals

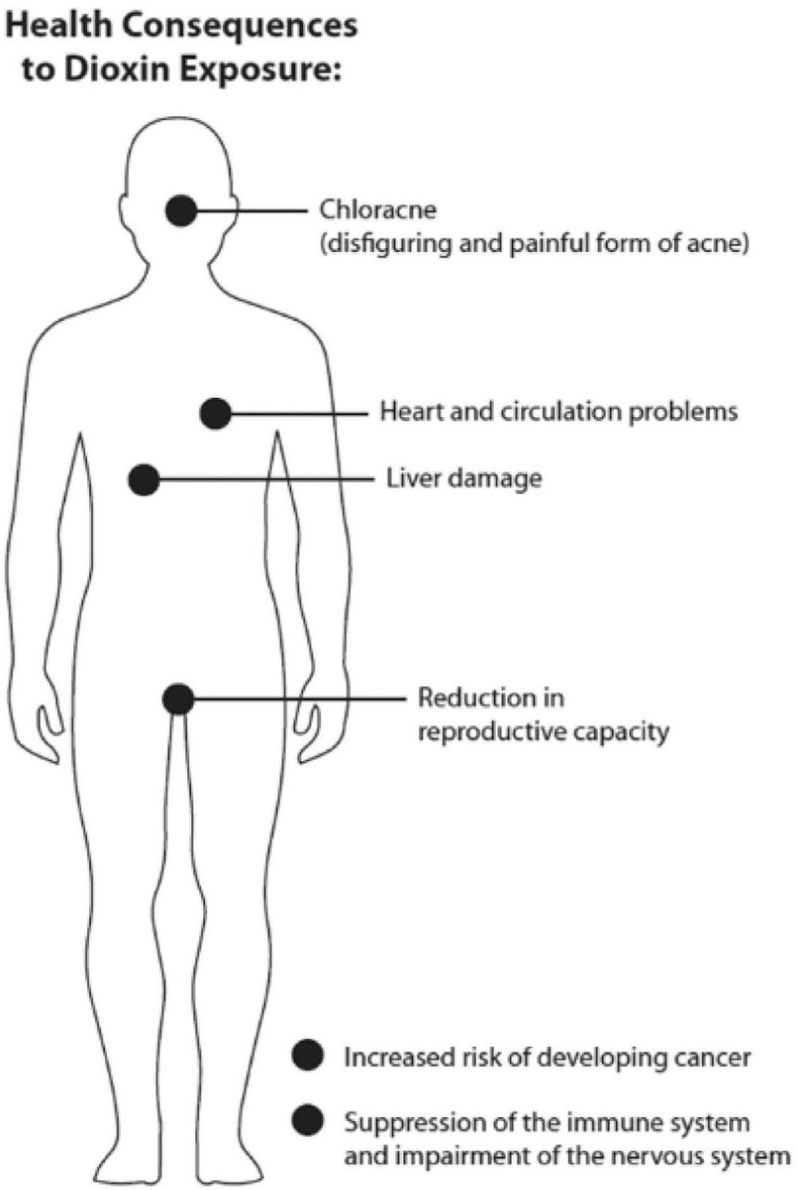


*Figure 1.7* Significant human health consequences of exposure to dioxin.

*Table 1.4* The interactive timeline showing the evolution of various regulatory measures to control or manage the pollution of dioxin in various environmental matrices.

| *Evolution of Regulatory Framework Specific to Dioxin* | | |
|---|---|---|
| *Dates* | *Guidelines/Regulations* | *Document Number* |
| February 1,1984 | EPA Releases Ambient Water Quality Criteria for 2,3,7,8-TCDD | EPA/440/5-84/007 |
| September 1, 1984 | EPA Releases Health Effects Assessment for 2,3,7,8-TCDD | EPA/540/1-86/044 |
| March 1, 1985 | EPA Releases Drinking Water Criteria Document for 2,3,7,8-TCDD | EPA/600/X-84/194-1 |
| September 1, 1985 | EPA Releases Final Health Assessment for Dioxins | EPA/600/8-84/014F |
| June l, 1994 | EPA Releases Draft Health Assessment for 2,3,7,8-TCDD and Related Compounds | EPA/600/BP-92/001c |
| September 1, 1994 | EPA Launches Dioxin Exposure Initiative (DEI) | |
| September 1, 1995 | EPA's Science Advisory Board Releases Report | EPA-SAB-EC-95-021 |
| September 1, 2000 | Science Advisory Board Releases Review Draft of the Dioxin Assessment | EPA/600/P-00/001 |
| May 1, 2001 | Science Advisory Board Releases Dioxin Reassessment | EPA-SAB-EC-01-006 |
| October 1, 2004 | EPA Releases ERD of Exposure and Human Health Reassessment of 2,3,7,8-TCDD | EPA/600/P-00/001Cb |
| July 1, 2006 | NAS Releases an Evaluation of the EPA Reassessment | N/A |
| September 1, 2008 | Interagency Dioxin Workgroup Releases Dioxins Q&A Website | N/A |
| February 1, 2009 | EPA Hosted 3-Day Public Dioxin Workshop | N/A |
| March 1, 2009 | Dioxins and the 2009 Science Plan | N/A |
| June 1, 2009 | EPA Releases the Dioxin Workshop Summary Report | N/A |
| September 1, 2009 | EPA Releases a Review of a University of Michigan study | EPA/600/R-10/038A |
| May 1, 2010 | EPA Releases the Reanalysis of Key Issues Related to Dioxin Toxicity | EPA/600/R-10/038A |
| January 1, 2011 | EPA Releases the Final Report on the Dioxin Toxicity Equivalency Factors | 100-R-l 0-005 |

for clean-up of the dioxin-contaminated soil media at various federal sites. Additionally, the Hazardous Air Pollutants for Hazardous Waste Combustors has established national emission standards for facilities that burn hazardous waste and release air pollutants like dioxins. Section 8(e) of the Toxic Substances Control Act and Clean Air Act requires any person who manufactures, processes, or distributes chemicals including dioxin has to inform the EPA.

## 10 Current status of dioxin

Both in Europe and North America, anthropogenic sources contributed to the unabated emission of PCDD/Fs in the 1930s and 1940s peaking around 1960s and 1970s. Treating the past emissions as a pulse input of PCDD/Fs in the atmosphere, current trends show declining emissions of dioxins in the atmosphere (Alcock and Jones, 1996). The atmospheric emissions of most common dioxins PCDD/Fs have continued to decline due to the implementation of various control measures. Interestingly, for the last two decades, the dioxin concentration levels have remained more or less asymptotic. This

concentration is still at above the threshold concentration – a concentration level at which there is no known harm to human health and ecology. Challenges still exist in the accurate quantitation of PCDD/Fs both in developed and developing countries. Although declining trends in the atmospheric concentration of PCDD/Fs in European air are observed, recently it was reported that European studies often underestimated the actual emissions and lacked better understanding of PCDD/Fs pollution in the Baltic Region (Assefa *et al*., 2014). Another worrisome aspect is the fact that the dioxins inventories were established almost two decades ago yet there is not sufficient information available regarding which specific source contributes to the dioxin in food that humans consume. Such understanding may be complicated by the presence of a multitude of dioxin sources as well as numerous congeners of dioxins. Nonetheless, this information is critical because 95% of human exposure to dioxins occurs through ingestion of dioxin-contaminated food (Weber *et al*., 2008).

Remediation of existing dioxin-contaminated sites needs to be pursued on a priority basis. Many studies have revealed that people living in proximity to the dioxin-contaminated sites have elevated levels of dioxins in their blood and milk. For example, a study reported that women living near open municipal dumpsites in India, Cambodia, Vietnam, and the Philippines during 1999–2000 had PCDD/F in their breast milk (Kunisue *et al*., 2004). The rapid growth in the semiconductor industry has also contributed to the new sources of dioxins. Recently, an air sampling study conducted at an electronic waste (e-waste) dismantling site located in Guiyu, the Guangdong Province, China has recorded the highest ambient atmospheric dioxin levels in the world (Li *et al*., 2007). It should be noted that e-waste typically contains chlorinated and brominated compounds, and improper disposal of such waste could potentially lead to the formation of dioxins. The e-waste dismantling facilities are typically located in developing countries where environmental regulations are lax and, hence, to reduce the global emission of dioxins, a better approach is needed whereby the burden of controlling and monitoring the air emissions through regulation should be the first priority before the facility can establish its operations.

Control and reduction in dioxins emissions are being pursued through various international conventions. For example, under the Stockholm Convention and the Convention on Long-Range Transboundary Air Pollution, a framework was established whereby the signatory nations aim at limiting and gradually reducing or eliminating the PCDD/Fs emissions (Idowu *et al*., 2013). Given the toxicity of dioxin, the USEPA has set the Maximum Contamination Level of 0.00003μg/L concentration of 2,3,7,8-TCDD for drinking water, whereas the Food and Drug Administration has recommended dietary restriction on consumption of fish and shellfish if they have a 2,3,7,8-TCDD concentration of 50 ng/L or more (USEPA, 2016; USFDA, 1981).The World Health Organization and Food and Agriculture Organization jointly issued maximum tolerable human intake levels of dioxins via food and these levels are currently used in the European Union (European Union, 2006)

Although a variety of mechanisms are in place to prevent, control, and mitigate the sources of dioxin emission, it is critical that a multi-pronged strategy is needed to control the atmospheric emission of dioxins. The emphasis should be not only on preventing the emissions from new sources but also to remediate the existing sources, identify sources that hitherto remained unnoticed, and evaluate the potential for new sources which might contribute to dioxin emissions. The effectiveness of dioxin mitigation measures primarily will rely on taking a holistic look that covers past, present, and future emissions.

## 11 Conclusions

Atmospheric emissions of dioxins such as PCDD/Fs is a critical area of research due to its persistence and adverse impact on human health and ecology. The majority of the emissions of dioxins arise from human activities. In the past, the growth of the chemical industry and uncontrolled combustion and incineration of solid waste has led to the emission of dioxins. Currently, emissions from incineration/combustion activities are mostly controlled; however, new emission sources, such as recycling of electronic waste and backyard fires, require implementation of control technologies to reduce dioxins emissions from these sources. The evolution of regulatory measures and emphasis on maximum available control technologies showed promising results in reducing dioxin emissions. While these developments largely remained confined to the developing countries, there is a need to focus on emissions of dioxins from developing countries where environmental regulations are often poorly enforced. Since dioxins are often carried farther from their source through well-documented LRAT of dioxins, controlling or managing emissions from developing countries is critical to maintaining a lower emissions inventory across the world.

## Acknowledgment

The author gratefully acknowledges Ms. Kelly Donovan, graphic artist at California State University, Fullerton, for assisting with the graphic illustration shown in Figures 1.2, 1.5 and 1.7.

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Chapter 2

# Atmospheric fate and transport of dioxins – persistent organic pollutants

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## 1 Introduction

Persistent Organic Pollutants (POPs) are the most toxic organic molecules and belong to mostly two subcategories: halogenated aromatic hydrocarbons and polycyclic aromatic hydrocarbons. They include industrial chemicals like polychlorinated biphenyls, pesticides like gamma-hexachlorocyclohexane, and by-products like dioxins (Ritter *et al*., 1995; Bajaj and Singh, 2015).

The word "dioxins" is broadly used for both dioxins and furans. They belong to structurally and chemically related complexes recognized as PCDDs (polychlorinated dibenzo para dioxins) and PCDFs (polychlorinated dibenzofurans) respectively (Lohmann and Jones, 1998; Fiedler *et al*., 2000; White and Birnbaum, 2009). They are extremely toxic and comprise 210 probable congeners with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) being the most noxious (Fiedler *et al*., 2000; Rumak *et al*., 2009). They are solely unintended by-products and are ubiquitous in the environment (ATSDR, 1998). Some dioxin-like PCBs (polychlorinated biphenyls) share common properties with dioxins and are usually included under the term "dioxins" (Fernandez-Gonzalez *et al*., 2015). They are the result of commercial production and employed for various purposes like capacitors, dielectric solutions, etc. (Korhonen *et al*., 2016). Although the usage of such organic compounds has been regulated and banned since the 1970s, their widespread usage and persistence has instigated excessive environmental pollution (Bajaj and Singh, 2015). For the purpose of simplicity, PCDDs, PCDFs, and dioxin-like PCBs will be frequently mentioned as dioxins all through the chapter.

The primary cause of dioxin pollution is anthropogenic activities (White and Birnbaum, 2009), though phenomenon such as volcano emissions are natural in origin and also contribute (Schecter *et al*., 1994). Earlier industrial activities were the foremost contributors of dioxins in the environment. Since regulatory measures have been implicated for many responsible processes, today uncontrolled incineration of domestic waste is of huge concern (White and Birnbaum, 2009). Dioxins are of great interest due to their adverse health effects and their persistency in the environment (Rumak *et al*., 2009). Exposure of humans to dioxins occurs generally through contaminated food either by directly ingesting the dioxin-contaminated crops or by ingesting other organisms that have bioaccumulated these organic compounds in their adipose tissue via the food chain (US EPA, 1994; ATSDR, 1998).

This chapter offers a broad outline of the identified sources of dioxins, the atmospheric fate of these toxic compounds, and in what way these complexes move in the various

compartments of the environment. Finally, the vulnerability of cold habitats towards adverse effects of dioxins due to their far-off movement to the cold environments is discussed.

## 2 Dioxins as Persistent Organic Pollutants (POPs)

Dioxins are a member of a collection of compounds known as POPs, organic compounds that are extremely noxious and persistent in the environment. Due to the ability to resist degradation and the long half-lives in soils, sediments, water, air, and biota, they are considered to be persistent in the environment. Being hydrophobic and lipophilic in nature, they have a property to accumulate through the food web by the process of biomagnification, posing a threat to living organisms and the environment (Jones and Voogt, 1999, Safe *et al*., 2012; Kanan and Samara, 2018). They can reach far-off from their site of production, making them an issue of global concern (Simonich and Hites, 1995; Bajaj and Singh, 2015). Considering these reasons, an international treaty named the Stockholm Convention became effective in 2004, aimed at protecting human and environmental health by checking and cutting POPs internationally. Dioxins make a notable category mentioned as POP under the Stockholm Convention. The persistence of any compound is determined by the rate at which it is removed from the milieu by physical, chemical, or biological means like hydrolysis, photolysis, atmospheric oxidation, biodegradation, etc. Furthermore, a combination of compound-specific properties and environmental factors governs this degradation rate (Wania, 1998).

The extensive release of POPs in the environment over the past several years has led to its global distribution. The contamination of the environment has exposed many organisms to acute and chronic toxic effects of POPs (Bajaj and Singh, 2015). Some specific effects of POPs include allergies, hypersensitivity, and disruption of the endocrine, nervous, reproductive, and immune systems (White and Birnbaum, 2009). Moreover, they can also pose developmental and oncogenic effects (White and Birnbaum, 2009; Dopico and Gomez, 2015; Kanan and Samara, 2018).

## 3 Dioxins in the environment

Dioxins enter the environment by both natural as well as anthropogenic means. The chief sources of dioxins released on to the earth are combustion, industries, waste incineration, and reservoirs of dioxins. Waste incineration of sewage sludge, hospital waste, municipal waste, etc. is the principal source of dioxin discharge in the environment (Clement *et al*., 1985; Zook and Rappe, 1994; Williams, 2005). Natural sources like volcanic eruptions, forest fires, building fires, etc. and anthropogenic sources like the burning of fuels (wood, coal, etc.) further contribute to the contamination of the environment by dioxins and dioxin-like compounds (EPA, 2000)

Dioxins are produced during the combustion of organic compounds in the presence of chlorinated compounds either due to the incomplete combustion of contaminants or due to the formation of dioxins by precursor or de novo synthesis pathways (Addink *et al*., 1995; EPA, 2000). Most of the dioxins released through combustion are deposited into the air (ATSDR, 1998). The EPA draft inventory on the basis of sources of polychlorinated dibenzofuran and polychlorinated dibenzo-p-dioxin specified that the contribution of domestic waste in dioxin pollution is significantly high (EPA, 1998).

Dioxins are released in the environment by metal smelting and refining operations (Lahl, 1993; Lahl, 1994; EPA, 2000), the manufacture of halogenated organic chemicals, and wood pulp bleaching during paper manufacturing (Clement *et al*., 1989; EPA, 1991). Some of the potential sources are coke production and iron sinter production during primary ferrous metal smelting and electric arc furnaces used during secondary ferrous metal smelting and refining (Lahl, 1993; Lahl, 1994; EPA, 2000). Printing inks, dyes, pigments, and polyvinyl chloride are the halogenated compounds associated with the release of dioxins and dioxin-like compounds in the environment (Zhang *et al*., 2015; Rodenburg, 2015). Although the production of PCBs showing dioxin-like activity has been banned in several countries, certain environmental origins still persist. The potential sources of coplanar PCBs in the environment include waste disposal and combustion of PCB contaminated products, disposal and continued use of products like capacitors, transformers, etc., and recycling of products contaminated with PCBs (NRC, 2001; Bajaj and Singh, 2015).

Dioxins generated in the past constitute the reservoir sources of dioxins. They are responsible for global dioxin exposure. The reservoirs can be categorized as short-term, intermediate, and long-term sources. Dioxins can be liberated from their reservoirs by particulate and vapor deposition, volatilization, and through ingestion by living organisms, including humans (Institute of Medicine, 2003). Dioxin contamination of geological formations constitutes static and long-term sources of its pollution. These sources can be reactivated and re-circulated into the environment by activities like mining, as was the case with ball clay deposits (Hayward *et al*., 1999). Soils and sediments in aquatic systems contribute as intermediate reservoirs of dioxin contamination. Here dioxins can remain for years with restricted degradation. The dioxins from these sources can directly enter the organisms through the food supply (Institute of Medicine, 2003). The short-term reservoirs are atmospheric suspension that remain in a volatilized state and are carried till deposited as rainfall. Another most dynamic short-term reservoir of dioxins is deposition in the adipose tissue of living organisms (Institute of Medicine, 2003). Microbial biotransformation (Cramer *et al*., 1995; Horstmann and McLachlan, 1995; EPA, 2000) and photolysis of chlorinated compounds (Vollmuth *et al*., 1994; Waddell *et al*., 1995; EPA, 2000) can result in the formation of dioxins under specific environmental settings.

Once released in the environment from various sources (Figure 2.1), dioxins first deposit onto various compartments of the environment and then accumulate over time due to their slow degradation and high persistence. Dioxins are deposited in the atmosphere as gaseous residues; however, deposition on land and water occur when released in liquid state. Dioxins may accumulate in any environmental compartment and can deposit onto biota. The major fraction of all dioxins released has been reported to deposit into the atmosphere (Fries, 1995; EPA, 2006).

## 4 Fate of dioxins in the atmosphere

As mentioned, once released from the source, a high proportion of dioxins enters the atmosphere. From the atmosphere, a major proportion of dioxins eventually reaches the Earth's surface, making it a major route of exposure to living organisms, particularly human beings. Figure 2.2 describes the multidirectional movement of dioxins between media. To track the deposition of dioxins from the atmosphere, a number of studies were

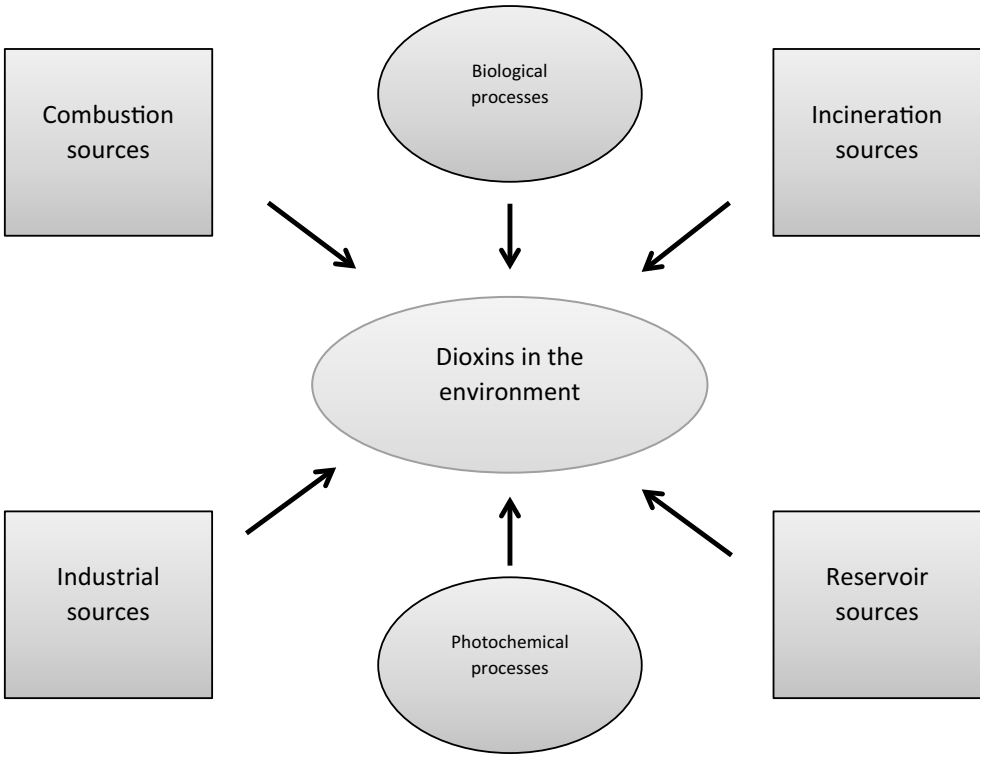


*Figure 2.1* Environmental sources of dioxins.

conducted which concluded that dioxin levels for a particular location could not be predicted using a mass balance equation (Brzuzy and Hites, 1995; Baker and Hites, 2000).

Dioxins are highly resilient to degradation in the environment and are fairly stable complexes. In the troposphere, photooxidation emerges to be the predominant process for the transformation of dioxins. Both particle phase and vapor phase dioxins appear to undergo photooxidation in the atmosphere through hydroxyl radical reaction. The rate of photooxidation in the atmosphere depends on factors like the temperature of the atmosphere and chlorination in dioxins. It appears to decrease with increasing dioxin chlorination and increase with the increase in temperature (in summers). The reaction of vapor phase dioxins with nitrate and ozone indicated that their atmospheric lifetime was 5 days and more than 330 days respectively. Other studies also indicated that the atmospheric lifetimes vary among dioxins. It varies from 39 days for octachlorodibenzofurans, 10 days for octachlorodibenzo-p-dioxins (OCDD), 2 days for 2,3,7,8-TCDD and 0.5 days for monochlorodibenzo-p-dioxins. The atmospheric half-life varies from more than 94 days for heptachlorinated congeners and 11 days for tetrachlorinated congeners of vapor-phase PCBs (EPA, 2000).

Photolysis is another process by which vapor phase dioxins appear to undergo transformation in the air through photodechlorination from high to low chlorinated congeners. The rate of photolysis declines with the intensification in number of chlorine atoms in vapor

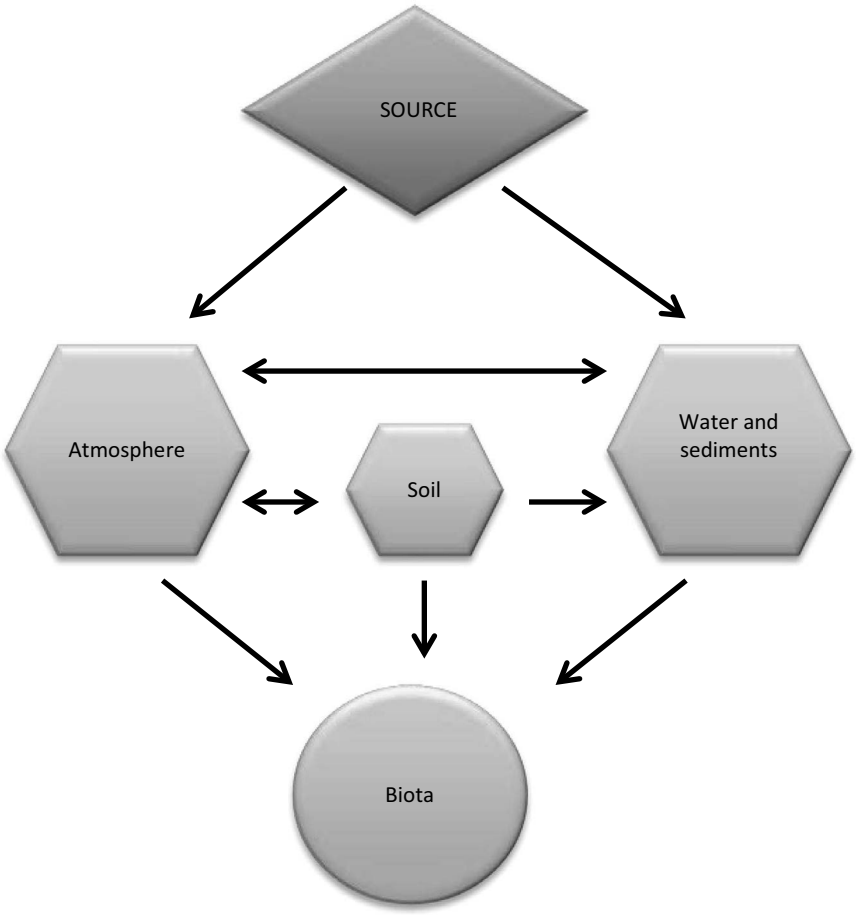


*Figure 2.2* Transport of dioxins between various environmental compartments.

phase dioxins. But results are different in case of dioxin-like PCBs, where the rate of photolysis increases with the increase in chlorination of the congener. Less chlorinated PCBs showed more resistance to photodechlorination. However, photodechlorination of particulate bound dioxins is highly slow. The rate of photolysis under actual environmental settings could be dissimilar since the most of these findings have been accompanied under laboratory conditions (EPA, 2000).

The degree to which they are degraded in the atmosphere by hydroxyl radicle reaction or photolysis is actually an unidentified aspect about dioxins' behavior. If the rates of deposition of dioxins on the surface of earth were faster than their depletion rates in the atmosphere, then the proportion that will reach the terrestrial food chains would be more (Institute of Medicine, 2003). Moreover, dioxins are not only dispersed locally but, on a global scale, they have a strong potential of long-range transport (Wania and Mackay, 1996; Van Pul *et al*., 1998).

## 5 Transport of dioxins

### 5.1 *Transport in air*

Various factors are involved in the atmospheric distribution of any pollutant, such as atmospheric temperature, particulate concentration, and particular properties of atmospheric contaminants such as the air-water partition coefficient (i.e., Henry's Law constant), vapor pressure, molecular diffusivity, melting point, and absorptivity (Jorgensen, 2010; Vallero, 2015). The pollutant can exist in two forms in the atmosphere: vapor phase and particle bound phase. The extent of chlorination decides the vapor pressure, which increases with the increase in chlorination of the organic compound. Lower vapor pressure, less particulate concentration, and high atmospheric temperature favor vapor phase; however, the particulate bound phase is coupled with higher vapor pressure and more chlorinated dioxins (i.e. pentachlorinated and more) (Bobet *el al*., 1990; Buckley-Golder *et al*., 1999; Institute of Medicine, 2003; Jorgensen, 2010). Both vapor and particulate bound phases exist for dioxins with intermediate vapor pressure, such as TCDD. Dioxins that exist in vapor phase are more volatile and tend to travel long distances in the atmosphere, for example dioxin-like PCBs (ATSDR, 1998; Institute of Medicine, 2003; Jorgensen, 2010).

Dry and wet depositions are the two modes by which dioxins leave the atmosphere and get deposited onto water, soil, or vegetation. The particulate bound dioxins leave the atmosphere mainly by wet deposition either precipitated with particles or suspended in water. Also, more chlorinated dioxins get adsorbed to the particles and deposited due to gravity via dry deposition. However, dioxins associated with the vapor phase leave the atmosphere due to diffusion and turbulence and get deposited directly onto vegetation cover (Fries, 1995; ATSDR, 1998).

### 5.2 *Transport in soil*

Dioxins are semivolatile organic compounds, mainly those having less chlorination. Thus, they can volatilize from the surface and re-enter the atmosphere, operating as inputs from tropospheric sources as reported in the Great Lakes (EPA, 2000). Dioxins can leave the soil mainly by soil erosion and runoffs into aquatic systems. Once bound to the soil particles below the surface, dioxins become immobilized and their movement becomes dependent on the carrier (e.g. oil), which may facilitate the movement through soil via diffusion. Thus, groundwater contamination via dioxins is unlikely in the absence of any carrier (as may occur at an oil spill site). However, this is not true in case of dioxin-like PCBs because leaching through soil may occur specifically in soils with low organic content (Weber *et al*., 2008).

There are reports describing the fate and transformation of dioxins for different environmental compartments on the basis of models. These models describe the environmental concentrations of dioxins and mention soil as major reservoir source for dioxins (Suzuki *et al*., 1998; Sinkkonen and Paasivirta, 2000; Huyen *et al*., 2015). The fate of these compounds in soil largely depends on the degradation rates.

### 5.3 *Transport in water*

Aquatic systems get dioxin contamination both the from atmosphere through dry and wet depositions as well as from the soil through runoff and erosion. Dioxins have low water

solubility, thus only a slight proportion dissolve in water. The major proportion of dioxins settle down onto sediments and a minor proportion re-enter the atmosphere through volatilization (less chlorinated dioxins and dioxin-like PCBs) (Institute of Medicine, 2003; ATSDR, 1998).

Dioxins in sediments ultimately get concealed, although any phenomenon (biological activity, floods, etc.) that can result in agitation of the sediments can lead to their re-suspension. They can enter the aquatic flow again and can volatilize from the water column in the troposphere, which is true specifically for more volatile dioxin-like PCBs mainly in summers. These suspended sediments can travel long distances and can reach far beyond their source of contamination before landing back (ATSDR, 1998; Institute of Medicine, 2003).

## 6 Dioxin contamination in cold environments

A huge portion of the earth is cold, ranging from the Arctic to Antarctica and from mountains to cold deserts. Before pollution from POPs was first described in the 1970s, such remote locations were considered to be pristine. Thus, cold ecosystems are impacted with pollution like all other habitats. Tropospheric transport has been considered as the foremost cause for POPs' contamination in these locations; however, other modes also contribute, like migrating species of mammals and birds, tourist activities, and oceanic transport (Bajaj and Singh, 2015).

POPs share some properties like high persistence and semivolatility, thus can volatilize from the terrestrial and aquatic environments into the atmosphere. They can travel to the areas where they have never been laid or produced, prior to their re-deposition (dry or wet deposition). During the complete lifetime of POPs, they can enter several cycles of volatilization and re-deposition and travel fairly long distances. This phenomenon of pollutant migration is termed as "global distillation effect," which is accountable for the global dispersion of these contaminants (Figure 2.3). Due to the low temperature of cold areas, the cycle is interrupted and further volatilization is hindered, leading to their accumulation in comparatively high levels, thus making these remote locations the ultimate trap for POPs. The degradation of POPs becomes tremendously slow in such environments due to low temperature conditions. Being hydrophobic, they tend to concentrate in the adipose tissues of the species that are thriving in such locations. Organisms that occupy cold habitats usually have prolonged lifespan, show periodic activity, and show little detoxifying activity; thus, they are considered to be more vulnerable to the antagonistic impacts of POPs (Bajaj and Singh, 2015).

Reports have shown that the global distillation of dioxins has considerably affected cold habitats (Kumar *et al*., 2001; Zheng *et al*., 2012; Pan *et al*., 2013; Han *et al*., 2016). Kumar *et al*. (2001) identified dioxins, furans, and dioxin-like PCBs in the Arctic and Antarctic wildlife. Authors reported greater concentration of dioxins in all the samples examined from the Arctic and Antarctic and proposed global distillation as the mechanism for the contamination of the polar regions with dioxins. A study was conducted to analyze the impact of long-range transport on mono- to octa-chlorinated dioxins, which demonstrated that less-chlorinated dioxins have a great potential for long-range transport. Thus low-chlorinated dioxins pose higher risk to remote areas like cold habitats than high-chlorinated dioxins (Han *et al*., 2016). In another study, when altitudinal distribution of dioxins in soil

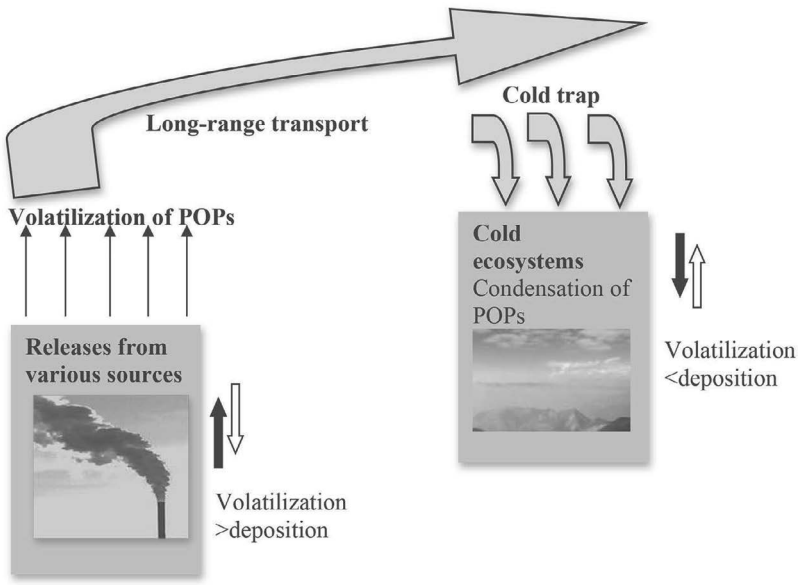


*Figure 2.3* Global distillation of POPs (volatilization, long-range transport and cold trap).

was determined, a rising trend of their concentration with altitude from alpine meadow samples was observed. This was accredited to the mountain cold-trapping effect (Zheng *et al*., 2012). Dioxin contamination of Tibet-Qinghai Plateau at high altitude is attributed to the long-range tropospheric transport (Pan *et al*., 2013). Thus, long-range transport of POPs like dioxins has notably affected both high-altitude alpine environments as well as high-altitude polar regions.

## 7 Conclusion

Dioxins have been widely distributed in the environment as persistent organic pollutants. They have also exhibited the ability to bioaccumulate in the adipose tissues of organisms. This chapter provides a comprehensive overview of the identified origins of dioxins and their fate in the atmosphere. Though dioxin production has been prohibited in numerous nations, certain origins still persist and those generated in the past constitute to the reservoir sources of dioxins, which are responsible for global dioxin exposure. Wastes from sewage sludge, hospital, municipal wastes, etc. are the principal sources of dioxin discharge in the environment. The dioxins from these origins can thus enter the biota through the food supply. After entering the environment, dioxins scatter among several environmental compartments. They follow a variety of common modes: they are present in both the vapor and particle bound phase in the atmosphere; however, they reach soil, water bodies, or vegetation by dry or wet deposition from the atmosphere. Dioxins are stable compounds and resist degradation in the environment. Degradation of dioxins slows down in soil and sediments; thus, they may endure for several ages when associated with soils and sediments. In the

atmosphere, the vapor phase undergoes degradation; however, degradation of particulate bound dioxins is highly slow. Long-distance transport of dioxins is evidenced by its presence in the Arctic and Antarctic wildlife, where it has never been used. The vulnerability of cold ecosystems to a prominent level of dioxins has become a subject of concern.

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Chapter 3

# Polychlorinated biphenyl in sediments of Subarnarekha River

## Levels, temporal and spatial distribution, feasible sources, and inventory

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## 1 Introduction

Polychlorinated biphenyl (PCB) is a special class chlorinated organic chemical which has been extensively used all over the world for its versatile characteristics. PCBs have remarkable physical properties, which include stability, noninflammability, low volatility, less solubility in water, and more solubility in hydrocarbon solvents. PCBs have been used commercially for a wide variety of purposes, including insulating fluids for electrical capacitors and transformers, plasticizers, hydraulic fluorides, heat transfer fluids, lubricants, and constituents of surface coatings and inks (Hutzinger *et al*., 1974; Gakuba *et al*., 2015). Among the large number of human-made organic chemicals, PCBs are usually considered chemicals in terms of environmental contaminants and toxicology with significant social impacts. Briefly, commercial PCBs are mixtures of isomers of chlorinated biphenyl homologs of varying degrees of chlorination. PCBs have been in commercial use since 1929. The residues of PCBs tend to accumulate and magnify in the food chain and lead to increasing speculation about their effect on the global ecology. After a series of mishaps, including several industrial accidents which resulted in direct contamination by PCBs in human and animal food sources, attention was focused on their potential health hazards, and many countries placed increasingly stringent restrictions on their use. The problem of environmental contamination by PCBs raised a serious alarm due to the persistence of their residues in the environment. PCBs are the main components of organic pollutants distributed worldwide. Most of these compounds accumulate in aquatic organisms and at lethal levels, finally reach human beings (Hutzinger, 1982; A Citizen Report, 1984).

Most of PCBs are neurotoxins, and their effect on animals is manifested through inhibiting or poisoning cholinesterase, a nerve tissue enzyme which is crucial to the orderly operation of the nervous system. According to Wang *et al*. (2015), these biologically active PCBs enormously speed up the process of natural eutrophication in water bodies, resulting in the blooming growth of nuisance algae known as phytoplankton, which alter, degrade, and cause severe problems to water quality. Furthermore, PCBs also have a strong affinity towards adipose tissue and they poison other parts of body. This information was brought to attention by Rachel Carson (Carson, 1962) in her well-known book called *Silent Spring*. PCB insertion into food chains and food webs was confirmed

through the biopsies of humans and animals at various places. "Biological Magnification" is the process of this accumulation of PCBs in the tissues of tolerant organisms at successively higher levels in a food chain. The toxic effects of PCBs to coastal areas were later described by Butler and Springer (1963) in their research.

### 1.1 *Routes of environmental pollution*

The concentration of PCBs was determined in the Mideast of the USA through the emissions from several landfills, incinerators of refuse, and sewage sludge. In the sanitary landfills, due to anaerobic fermentation together with other volatile organic materials, there is continuous emission of gaseous products into the environment. The data from projections based on the amount of methane generated annually from landfills and PCB to methane ratio of 0.3 μg $PCB_s/m^3$ found from the landfills sampled, indicates that the annual PCBs emissions from sanitary landfills in the USA is of the order 10–100 kg/year. The amount of PCBs from the incinerator stack emission ranged from 0.3–3 $ug/m^3$ and annual emissions per stack was 0.25 kg/year. When it is compared with the USA, annually, it is far more i.e. 900,000 kg PCBs/year cycle through the atmosphere (Murphy *et al*., 1985). In the USA, about 10 Tonnes/year has been used as formulations of scrap transformer fluid containing PCBs (WHO/EURO, 1988) and the effect of the use of unauthorised has led to the local contamination of milk supplies. Stehr *et al*., (1985) reported the possibility of impurity with PCBs of oils used by amateur radio operators and more than 50 mg/kg was found in two out of 77 oil samples.

### 1.2 *Relevance*

PCBs belong to the group of organic pollutants having hydrophobic properties, which resist the process of metabolism. These compounds accumulate in the tissues of organisms and get magnified in the higher trophic level through the food chain. PCBs find their way into the environment through various routes and finally sink into the sediments, thereby causing severe damage to the benthic organisms leading to ecological imbalance. The abiotic and biotic condition of the aquatic environment has greatly influenced the fate of PCBs. There are a total 209 congeners of PCBs, and 80–90 have been detected frequently in different compartments of the environment. It was observed that some of the congeners that have chlorine substitution occupy both Para position and Meta substituent and show high toxicity because of their co-planarity (Kannan *et al*., 1988). Although the use of PCBs in Europe and USA was restricted, they have been in use in large quantities in developing countries like India. The cumulative effects of the residues of such highly persistent organochlorine compounds on the environment can be expected to be considerable. Due to their indiscriminate application during previous decades, these compounds were found to be present in varying quantities in different compartments of the environment.

The work embodied in this research dealt with an exclusive evaluation of the level of contamination of various congeners of PCBs in sediments at different locations of the Subarnarekha River around Jamshedpur during the pre-monsoon, monsoon, and post-monsoon periods and the study their physicochemical properties.

## 2 Materials and methodology

### 2.1 *Description of sampling location*

Five sampling points were chosen at the bank of Subarnarekha River of Jamshedpur (shown in the map, Figure 3.1). The sites were located close to Bargidhi Ghat, mouth of the Mango Bridge drain joining Subarnarekha River, Adityapur Bridge, Mango Basti, and Adityapur Colony. All these sampling sites possess unique characteristics with regards to the nature of the pollutants they receive. The sampling points are at a distance of 2–3 kms from each other.

### 2.2 *Bargidhi Ghat (Site 1)*

The first sampling point is the entrance point of the Subarnarekha River in Jamshedpur. The waters of this region are relatively clean in comparison with other sampling sites, namely, Mango Bridge, Adityapur Bridge, and Mango Basti. However, due to the presence of a temple near to this ghat, various rituals were carried out regularly. Hence, a large amount of garbage has been added in the River water, almost every day, thus increasing the pollutant load in this region continuously.

### 2.3 *Mango Bridge (Site 2)*

This sampling point is one of the most polluted sites among all sampling sites. Out of 17 drains of Jamshedpur, the contribution of Mango Bridge drain in terms of volume and also in quantity of pollutants is significantly large. It alone contributes 53.6% of the total drainage flow. The percentage contribution of industrial wastes related to the total waste water from the Mango Bridge drain is 6.3%. The sediment samples were collected from a site where the drain meets Subarnarekha River. Due to the presence of a large amount of waste and litter, the area imparts an obnoxious odour during the pre- and post-monsoon period. Besides, during the pre-monsoon and post-monsoon period, agricultural activities are at their peak near the sampling point at the Mango Bridge drain. Similar activities are also observed on the other side of the River bank, which add substantially to pesticidal pollution.

### 2.4 *Adityapur Bridge (Site 3)*

Various human activities mark this sampling point. The areas around the sampling point are being polluted intensively by the slum dwellers. There is a marked increase in pollution during the pre- and post-monsoon period due to the agricultural activities.

### 2.5 *Mango Basti (Site 4)*

Beside Mango Basti, extensive wheat and vegetable farming take place, which inevitably use fertilizers and pesticides. Thus, pesticide residues find their way into the Subarnarekha River. It was also observed that waste materials, along with fly ash, were discharged into the river from the nearby thermal power site.

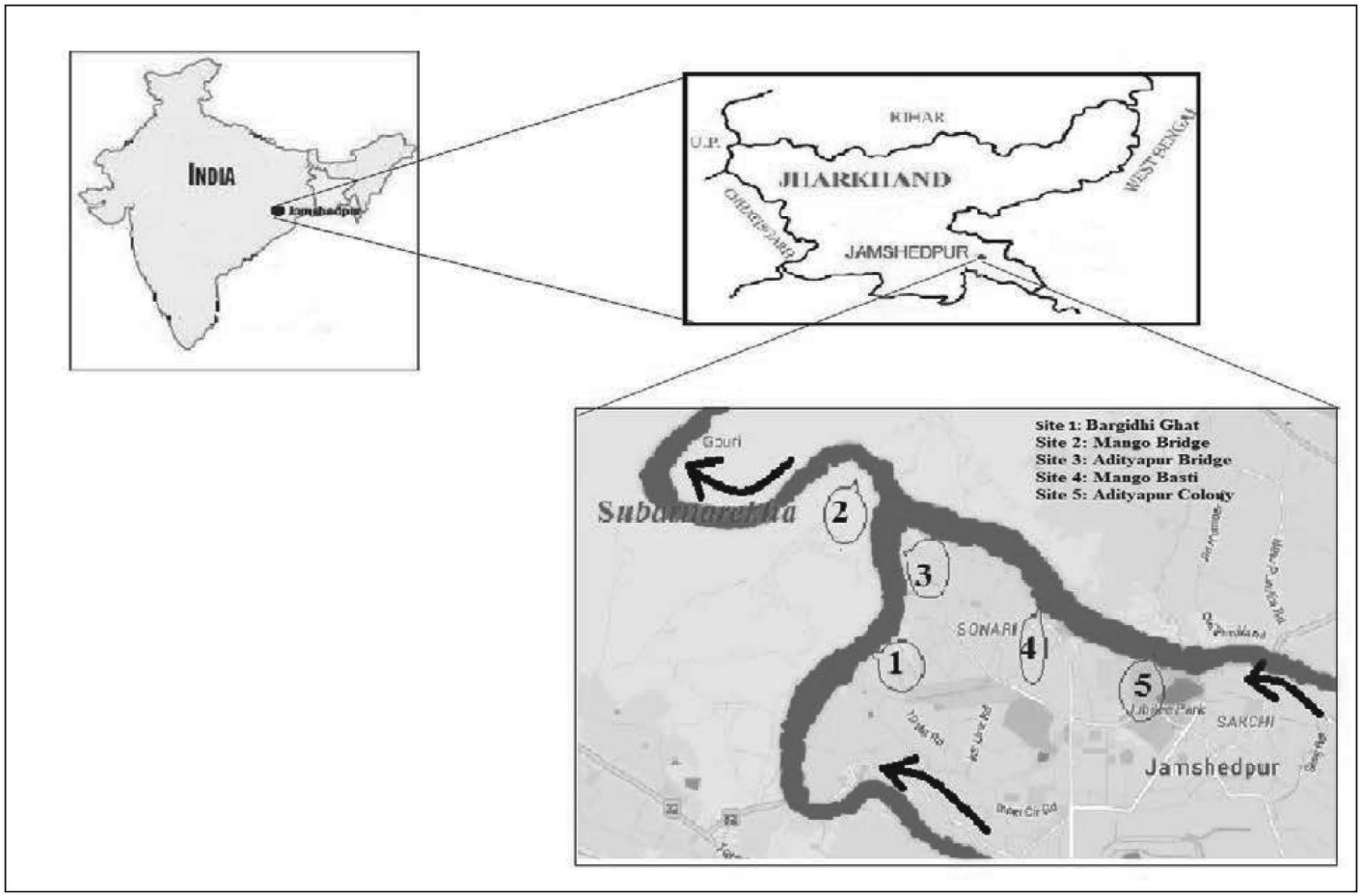


*Figure 3.1* Sampling site location for PCBs Congeners collection.

### 2.6 *Adityapur Colony (Site 5)*

The last sampling point is at Adityapur Colony. The water at this point is apparently cleaner than all other sampling sites. However, various activities of the human deed can be noticed in this area.

### 2.7 *Sediment extraction*

Surface sediments were collected from the bank of Subarnarekha River at five different locations viz., Bargidhi Ghat, Mango Bridge, Adityapur Bridge, Mango Basti, and Adityapur Colony around Jamshedpur (Figure 3.1). Immediately after collection of sediments in triplicate, they were stored in the deep freeze at −25°C to avoid degradation of the pollutants. After thawing and thorough mixing, about 40–50 g (wet weight) of sediments were spiked with 100 µl of 0.1 ppm IUPAC 88 as an internal standard and shaken overnight with 60–70ml Nano grade acetone for extraction of chlorinated hydrocarbons followed by one hour post extraction with 40–50 ml distilled hexane. The purpose of the addition of the internal standard was to check the recovery of the components of PCBs from the sediments. Both the extracts were combined and washed thoroughly with 50 ml bidistilled water to remove acetone from the solvent mixture and to transfer the extracted components of chlorinated compounds into the hexane layer, which was subsequently concentrated to 1 ml by a Kuderna-Danish evaporator and treated with activated copper powder to remove sulphur. The aliquots were then passed through a micro column of alumina for clean-up of the interfering agents present in the sediment extracts. The eluted extracts from the alumina column were concentrated to 1 ml by a Kuderna-Danish evaporator and passed through a silica column in order to separate the components of PCBs. The fractions of the elute from the silica column were concentrated to 1 ml in the same way and transferred quantitatively into sample vials and sealed with Teflon coated caps. Concentrations expressed on a dry weight basis. An aliquot of the original sample was dried overnight at 120°C and the fraction dry weight was determined.

## 3 Analysis

### 3.1 *Analysis of PCBs*

The identification and quantification of various congeners of PCBs from environmental samples are the most critical part of the analysis because of the presence of a number of co-eluting peaks in the chromatogram. For accurate determination of the level of contamination of PCB compounds in the riverine environment, it is of essential importance to evaluate the various steps of analysis, such as extraction of contaminants, their recovery, and elution patterns through chromatographic columns like alumina and silica columns. Moreover, pre-separation of PCBs from the contaminant extract is of utmost importance for identification of each of the isomers and metabolites of individual congeners of PCBs.

Gas chromatography (Perking Elmer 8700) coupled with a $Ni^{63}$ electron capture detector and capillary columns (SE-54) (I.d. 0.25 mm and 30 m length) have been used for the identification of PCB congeners. One microliter of each of the samples was injected in the capillary port of GC column. The oven temperature was programmed as follows: Initial temperature of 110°C for 2 min; increased at the rate of 10°C per min to 180°C and

hold for 8 min; raised at the rate of 4°C per min to 220°C and hold for 5 min; finally raised at the rate of 4°C/min to 270°C and hold for 15 min. The standby time was 2 mins. The detector temperature was 340°C. To check the reproducibility of the chromatogram 100 µl of 0.1 ppm, IUPAC-88 was also spiked into 0.9 ml standard solution of the PCB mixture containing 55 components and analysed by the GC under the same condition. The identification and quantitation of different individual congeners of PCBs in each of the samples were performed with respect to the peaks of the chromatogram of the standard mixture and the individual PCB congeners.

## 4 Results and discussion

### 4.1 *Physical and stereochemical properties of PCBs*

The chemical formula for PCBs represented as $C_{12}H_{10}Cl_n$, where n represents the number of chlorine atoms in the molecule and can be ranged from 1 to 10. The degree of substitution gives the PCBs relative molecular mass. Monochlorobiphenyl and completely chlorinated biphenyl ($C_{12}H_{10}Cl_{10}$) has a relative molecular mass of 188 and 494 respectively (US EPA, 1980). PCB congeners are odorless, colorless, and often found in crystalline compounds, but PCBs available commercially are various combinations of these congeners having a clean, faint yellow or dark colour. They do not crystallize at a lower temperature but turn into solid resins at an elevated temperature. Due to the presence of the chlorine atoms in the PCBs, their density is higher. PCBs have high flash points (170–380$^0$C) and are fire resistant. PCB vapors are heavier than air, but their mixtures with air do not give any explosive reaction. Their electrical conductivity is low and extremely to breakdown during thermal reaction. Due to these properties, they are extensively used as cooling liquids in electrical equipment (USEPA, 1980; WHO/EURO, 1987). They possess a variety of different chemicals as well as redundant and oxidants due to their chemical stability. According to laboratory experiments, they remain chemically unaffected in the oxidising medium or some active metals at higher temperatures (up to 170$^0$C) for undisturbed long periods (WHO/EURO, 1987). In water, PCBs are insoluble, whereas they dissolve easily in organic compounds, oil, and fat (WHO/EURO, 1987). Some of the physical and chemical properties for several of congeners are represented in (Table 3.1).

The investigation of sediment samples from different locations of the Subarnarekha River indicated the presence of 25 congeners of PCBs, out of which there were four dichlorobiphenyls (PCB-4, PCB-5, PCB-7, and PCB-11), one triochlorobiphenyl (PCB-29), two tetrachlorobiphenyls (PCB-49, PCB-81), seven pentachlorobiphenyls (PCB-156, PCB-123, PCB-116, PCB-114, PCB-105, PCB-126, and PCB 118), four hexachlorobiphenyls (PCB-157, PCB-153, PCB-138, and PCB-169), four heptachlorobiphenyls (PCB-182, PCB-181, PCB-185, and PCB-170), and two octachlorobiphenyls (PCB-199 and PCB-195). It clearly shows that the non-ortho-chlorinated biphenyls (PCB-14, PCB-11, PCB-126, and PCB-169) and the mono-ortho-chlorinated biphenyls (PCB-5, PCB-29, PCB-123, PCB-114, PCB-105, and PCB-118) possess the co-planarity because of their stereo chemically most stable configuration. The di-ortho-chlorinated biphenyls (PCB-4, PCB-81, PCB-49, PCB-156, PCB-116, PCB-153, PCB-138, and PCB-170), tri-ortho-chlorinated biphenyls (PCB-157, PCB-182, PCB-185, PCB-181, and PCB-195), and tetra-ortho-chlorinated biphenyls (PCB-199) are non-planar due to

*Table 3.1* Physical and chemical properties of detected congeners at various sampling sites.

| | *Molecular formula* | *Molecular Weight* | *CAS no.* | *Melting Point ($^0C$)* | *Boiling Point ($^0C$)* | *Density $g/cm^3$* | *Water Solubility (mg/l at 25°C)* | *Vapor Pressure (mm Hg at $25^OC$)* | *Henry's Law Constant (atm. $m^3$/mol at $25^0C$)* | *Log $K_{ow}$* | *Half-Lives Surface water ($t½$)* | *Configuration* | *IUPAC NAME* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PCB-4** | $C_{12}H_8Cl_2$ | 223.09 | 13029–08–8 | 60.50 | 312.0 | 1.05 | 1.50 | 0.133 | 30.20 | 4.97 | 34.5 d | Non- Planar | 2,2′ Dichlorobiphenyl |
| **PCB-5** | $C_{12}H_8Cl_2$ | 223.09 | 16605–91–7 | 28.00 | 172.0 | 1.05 | 1.70 | 0.151 | 19.56 | 4.99 | 4–11 d | Co-Planar | 2,3-Dichlorobiphenyl |
| **PCB-7** | $C_{12}H_8Cl_2$ | 223.09 | 33284–50–3 | 24.1–24.4 | 167.0 | 1.05 | 1.40 | 0.179 | 28.6 | 28.6 | 4–11 d | Co-Planar | 2,4-Dichlorobiphenyl |
| **PCB-11** | $C_{12}H_8Cl_2$ | 223.09 | 2050–67–1 | 29.00 | 320.0 | 1.05 | 1.05 | 0.026 | 13.58 | 5.30 | – | Co-Planar | 3,3′-Dichlorobiphenyl |
| **PCB-14** | $C_{12}H_8Cl_2$ | 223.09 | 34883–41–5 | 36.00 | – | – | 1.05 | 0.078 | 16.72 | 5.63 | – | Co-Planar | 3,5-Dichlorobiphenyl |
| **PCB-29** | $C_{12}H_7Cl_3$ | 257.54 | 15862–07–4 | 78.50 | – | 1.14 | 0.14 | 0.011 | 20.27 | 5.77 | 31–36 min | Co-Planar | 2,4,5-Trichlorobiphenyl |
| **PCB-49** | $C_{12}H_6Cl_4$ | 291.98 | 41464–40–8 | 66.50 | – | – | 0.016 | 0.0070 | 20.27 | 5.73 | 4–11 d | Non- Planar | 2,2′,4,5′-Tetrachlorobiphenyl |
| **PCB-52** | $C_{12}H_6Cl_4$ | 291.98 | 35693–99–3 | 87–89 | 345.0 | – | 0.046 | 0.0073 | 22.29 | 6.26 | 19.7 d | Non- Planar | 2,2′,5,5′-Tetrachlorobiphenyl |
| **PCB-105** | $C_{12}H_5Cl_5$ | 326.43 | 32598–14–4 | 103.0 | – | 1.24 | 0.040 | 0.00070 | 5.68 | 6.41 | – | Co-Planar | 2,3,3′,4,4′-Pentachlorobiphenyl |
| **PCB-114** | $C_{12}H_5Cl_5$ | 326.43 | 74472–37–0 | 99.00 | 374.0 | | 0.012 | 0.0055 | 6.99 | 6.45 | – | Co-Planar | 2,3,4,4′,5-Pentachlorobiphenyl |
| **PCB-116** | $C_{12}H_5Cl_5$ | 326.43 | 18259–05–7 | 123.50 | 381.0 | 1.28 | 0.020 | 0.0034 | 18.34 | 6.85 | – | Non- Planar | 2,3,4,5,6-Pentachlorobiphenyl |
| **PCB-118** | $C_{12}H_5Cl_5$ | 326.43 | 31508–00–6 | 106.0 | – | – | 0.013 | 0.00016 | 8.61 | 6.57 | – | Co-Planar | 2,3′,4,4′,5-Pentachlorobiphenyl |
| **PCB-123** | $C_{12}H_5Cl_5$ | 326.43 | 65510–44–3 | 125.0 | 389.0 | – | 0.002 | 0.00099 | 8.80 | 6.74 | 21.2 min | Co-Planar | 2,3′,4,4′,5′-Pentachlorobiphenyl |
| **PCB-126** | $C_{12}H_5Cl_5$ | 326.43 | 57465–28–8 | 106.0 | – | – | 0.032 | 0.00002 | 8.39 | 6.38 | 304 min | Co-Planar | 3,3′,4,4′,5-Pentachlorobiphenyl |
| **PCB-138** | $C_{12}H_4Cl_6$ | 360.87 | 35065–28–2 | 79.00 | | 1.34 | 0.0159 | 0.000005 | 7.60 | 7.90 | 8.2 h | Non- Planar | 2,2′,3,4,4′,5′-Hexachlorobiphenyl |

(*Continued*)

*Table 3.1* (Continued)

| | *Molecular formula* | *Molecular Weight* | *CAS no.* | *Melting Point ($^0C$)* | *Boiling Point ($^0C$)* | *Density $g/cm^3$* | *Water Solubility (mg/l at 25°C)* | *Vapor Pressure (mm Hg at $25^OC$)* | *Henry's Law Constant (atm. $m^3$/mol at $25^0C$)* | *Log $K_{ow}$* | *Half-Lives Surface water ($t½$)* | *Configuration* | *IUPAC NAME* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PCB-153** | $C_{12}H_4Cl_6$ | 360.87 | 35065–27–1 | 103.5 | 324.0 | – | 0.0012 | 0.000680 | 12.46 | 12.46 | 25–53 min | Non- Planar | 2,2′,4,4′,5,5′-Hexachlorobiphenyl |
| **PCB-156** | $C_{12}H_4Cl_6$ | 360.87 | 38380–08–4 | 127.0 | – | – | 0.0053 | 0.000809 | 17.53 | 7.13 | – | Co-Planar | 2,3,3′,4,4′,5-Hexachlorobiphenyl |
| **PCB-157** | $C_{12}H_4Cl_6$ | 360.87 | 69782–90–7 | 141.0 | 394.0 | 1.25 | 0.015 | 0.000139 | 3.30 | 7.18 | – | Co-Planar | 2,3,3′,4,4′,5′-Hexachlorobiphenyl |
| **PCB-169** | $C_{12}H_4Cl_6$ | 360.87 | 32774–16–6 | 201.0 | – | – | 0.00005 | 0.0000108 | 1.57 | 7.42 | 548 min | Co-Planar | 3,3′,4,4′,5,5′-Hexachlorobiphenyl |
| **PCB-170** | $C_{12}H_3Cl_7$ | 395.32 | 35065–30–6 | 135.0 | 387.0 | – | 0.0076 | 0.00372 | 1.52 | 7.05 | – | Non- Planar | 2,2′,3,3′,4,4′,5-Heptachlorobiphenyl |
| **PCB-181** | $C_{12}H_3Cl_7$ | 395.32 | 74472–47–2 | 140.0 | – | 1.35 | 0.0062 | 0.000644 | 40.83 | 7.14 | – | Non- Planar | 2,2′,3,4,4′,5,6-Heptachlorobiphenyl |
| **PCB-182** | $C_{12}H_3Cl_77$ | 395.32 | 60145–23–5 | 152.0 | – | – | 0.00534 × | 0.000131 | 26.04 | 7.22 | – | Non- Planar | 2,2′,3,4,4′,5,6′-Heptachlorobiphenyl |
| **PCB-185** | $C_{12}H_3Cl_7$ | 395.32 | 52712–05–7 | 149.0 | – | 1.37 | 0.00047 | 0.0000475 | 1.62 | 7.93 | – | Non- Planar | 2,2′,3,4,5,5′,6-Heptachlorobiphenyl |
| **PCB-195** | $C_{12}H_2Cl_8$ | 429.76 | 52663–78–2 | 176.0 | – | – | 0.00334 | 0.000112 | 1.11 | 7.49 | – | Non- Planar | 2,2′,3,3′,4,4′,5,6-Octachlorobiphenyl |
| **PCB-199** | $C_{12}H_2Cl_8$ | 429.76 | 52663–75–9 | 170.0 | – | 1.36 | 0.00323 | 0.000320 | 1.01 | 7.51 | – | Non- Planar | 2,2′,3,3′,4,5,5′,6′-Octachlorobiphenyl |

Melting Point (°C): Hutzinger *et al.*, 1974

Boiling Point (°C): Mackay *et al.*, 1982; Shiu and Mackay, 1986

Water Solubility ($g/m^3$ or mg/L at 25°C): shake flask-GC/ECD, Hutzinger *et al.*, 1974

Vapor Pressure: Knudsen-effusion technique, extrapolated from 37–54.92°C, Smith *et al.*, 1964

Henry's Law Constant: Murphy *et al.*, 1987

Octanol/Water Partition Coefficient, log $K_{OW}$: Hansch *et al.*, 1995

Half-Lives Surface water ($t½$): Neely *et al.* (1983)

steric hindrance between the chlorine atoms at the ortho-position (Tanabe, 1988). The toxic potential of various congeners of PCBs is very much related to the degree of chlorination as well as the co-planarity. The highly chlorinated congeners of PCBs like PCB-185, PCB-181, PCB-182, PCB-199, PCB-170, and PCB-19 are more toxic than the low chlorinated congeners like PCB-4, PCB-7, PCB-14, PCB-5, PCB-11, PCB-29, etc. However, due to the stereo-chemical configuration, the location of atoms of chlorine with a biphenyl ring was found to be significant. The structural activity relationship studies indicated that co-planar PCBs are far more toxic than the non-coplanar PCBs because of their structural fit into the body tissues leading to physiological disorders (Boon *et al*., 1987; Kannan *et al*., 1988). Among the co-planar congeners of PCBs, PCB-126 and PCB-169 were found to be the most toxic components because of their structural configuration as well as the electronic effect of the atoms of chlorine present at the meta and para positions. The most significant observation of this study is the detection of highly persistent and toxic congeners of PCBs at different sampling sites along the Subarnarekha River. The most toxic congener of PCBs detected at different locations are the non-ortho-chlorinated congeners, namely PCB-126 (3,3',4,4',5-pentachlorobiphenyl) and PCB-169 (3,3',4,4',5,5' hexachlorobiphenyls and the mono-ortho-chlorinated congener PCB-114 (2,3,4,4',5-pentachlorobiphenyl). The toxicity of these components is significant. Coplanar PCB congeners have serious toxic biological effects on human beings, such as dermal disorder, body weight loss, thymic atrophy, reproductive toxicity, hepatic damage, immune toxicity, teratogenicity, high binding affinity to hepatic cytosolic receptor protein, and great induction potency of 3-methylcholanthrene type hepatic microsomal enzymes (Aulerich *et al*., 1985; Ghaeni *et al*., 2015; Gioia *et al*., 2014; Chakraborty *et al*., 2016; Cui *et al*., 2016; Toan & Quy 2015; Mahmood *et al*., 2014). The toxic potentials of such co-planar congeners of PCBs are closely related to those of the corresponding tetrachlorodibenzo-p-dioxin (TCDD) as is evident from the studies of Tanabe (1985).

A detailed discussion on the seasonal variation in the concentration and distribution of several congeners of $PCB_s$ illustrated below.

## 5 Levels of contamination during the pre-monsoon

### 5.1 *Bargidhi Ghat sampling site (Site 1)*

Sediments from Bargidhi Ghat showed a wide range of variations in the concentration of PCBs during the pre-monsoon (Table 3.2 and Figure 3.2). At the Bargidhi Ghat, only ten congeners of PCBs viz., PCB-4, PCB-7, PCB-14, PCB-5, PCB-29, PCB-156, PCB-123, PCB-116, PCB-182, and PCB-126 were detected. Among these, PCB-4, PCB-156, PCB-116, and PCB-182 were non-planar, while PCB-7, PCB-14, PCB-5, PCB-29, PCB-123, and PCB-126 were co-planar. The concentration of total PCB congeners detected at the Bargidhi Ghat sampling site was 4.94 ng/g during the pre-monsoon period. The most predominant congeners of PCBs detected at the Bargidhi Ghat sampling site were found to be PCB-5 (2,3- dichloro-biphenyl), PCB-116, PCB-156, and PCB-4. Among the ten congeners of PCBs detected, PCB-5 constitutes the 34.65% of total PCBs, while PCB-116, PCB-156, and PCB-4 were composed of 28.22%, 17.68%, and 9.89% respectively, indicating the abundance of different congeners of PCBs.

*Table 3.2* Average seasonal variation in concentration of various congeners at the sampling site 1.

| *PCB Congeners* | *Average Concentration (ng/g) with Standard Deviation* | | | *% Concentration in ng/g* |
|---|---|---|---|---|
| | *Pre-Monsoon* | *Monsoon* | *Post-Monsoon* | |
| CB-4 | 0.580 ± 0.014 | 0.440 ± 0.006 | 0.278 ± 0.002 | 9.58 |
| CB-7 | 0.170 ± 0.002 | 0.160 ± 0.006 | 0.151 ± 0.001 | 3.55 |
| CB-14 | 0.200 ± 0.028 | 0.180 ± 0.009 | 0.159 ± 0.002 | 3.98 |
| CB-5 | 1.810 ± 0.029 | 1.170 ± 0.016 | 0.626 ± 0.008 | 26.63 |
| CB-29 | 0.410 ± 0.008 | 0.310 ± 0.029 | 0.216 ± 0.001 | 6.91 |
| CB-156 | 0.990 ± 0.029 | 0.670 ± 0.034 | 0.390 ± 0.003 | 15.14 |
| CB-123 | 0.160 ± 0.008 | 0.154 ± 0.001 | 0.147 ± 0.004 | 3.40 |
| CB-116 | 1.450 ± 0.058 | 0.980 ± 0.019 | 0.540 ± 0.005 | 21.93 |
| CB-182 | 0.180 ± 0.008 | 0.410 ± 0.018 | 0.153 ± 0.0020 | 5.49 |
| CB-126 | 0.160 ± 0.009 | 0.153 ± 0.008 | 0.146 ± 0.008 | 3.39 |

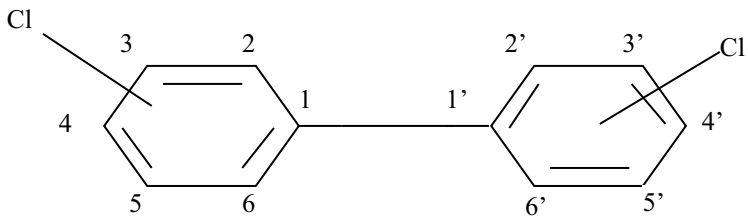


*Figure 3.2* Skeleton of Polychlorinated Biphenyl.

## 5.2 *Mango Bridge sampling site (Site 2)*

Table 3.3 and Figure 3.3 show the concentrations of various congeners of PCBs at the Mango Bridge sampling site. The predominant congener of PCBs detected at this site during the pre-monsoon period was found to be PCB-156 (2,2',4,5,5'-pentachlorobiphenyls) (408.88 ng/g), constituting 85.922% of total PCBs. The concentration of total PCBs during the pre-monsoon period at the Mango Bridge sampling site was estimated to be 475.868 ng/g. Altogether, 18 congeners of PCBs (viz. PCB-4, PCB-7, PCB-5, PCB-11, PCB-29, PCB-81, PCB-49, PCB-156, PCB-123, PCB-116, PCB-114, PCB-182, PCB-126, PCB-118, PCB-199, PCB-170, and PCB-195) were detected at the Mango Bridge sampling site. PCB-156 alone contributed a remarkably high percentage (85.992%), while the rest contributed only 0.30 to 3.099%. Among the PCB congeners detected, PCB-4, PCB-81, PCB-49, PCB-1 01, PCB-116, PCB-157, PCB-182, PCB-199, PCB-170, and PCB-195 are of non-planar configuration, while the other congeners like PCB-7, PCB-5, PCB-11, PCB-29, PCB-123, PCB-114, PCB-126, and PCB-118 were of co-planar configuration, indicating the relative distribution pattern.

## 5.3 *Adityapur Bridge sampling site (Site 3)*

The concentration of several congeners of PCBs detected was in the range of 0.141 ng/g to 0.78 ng/g. The lowest concentration was found to be 0.141 ng/g for PCB-157. Altogether, 21 PCB congeners were detected during the pre-monsoon season at the Adityapur Bridge

*Table 3.3* Average seasonal variation in concentration of various congeners at the sampling site 2.

| *PCB Congeners* | *Average Concentration (ng/g) with Standard Deviation* | | | *% Concentration in ng/g* |
|---|---|---|---|---|
| | *Pre-Monsoon* | *Monsoon* | *Post-Monsoon* | |
| CB-4 | 0.93±0.006 | 1.32±0.025 | 0.28±0.002 | 0.15 |
| CB-7 | 0.93±0.008 | 1.32±0.015 | 0.28±0.002 | 0.15 |
| CB-14 | 16.14±0.150 | 24.6±0.250 | 3.24±0.037 | 2.62 |
| CB-5 | 14.34±0.120 | 21.39±0.280 | 2.84±0.032 | 2.29 |
| CB-29 | 0.61±0.008 | 0.92±0.015 | 0.228±0.0011 | 0.10 |
| CB-81 | 14.84±0.150 | 422.44±0.300 | 2.94±0.033 | 26.15 |
| CB-49 | 3.64±0.090 | 5.46±0.080 | 0.81±0.008 | 0.59 |
| CB-156 | 408.94±6.50 | 610.59±10.50 | 77.68±0.950 | 65.17 |
| CB-123 | 11.54±0.250 | 17.37±0.250 | 2.3±0.025 | 1.85 |
| CB-116 | 0.87±0.005 | 1.24±0.025 | 0.285±0.002 | 0.14 |
| CB-157 | 2.04±0.030 | 3.04±0.050 | 0.48±0.004 | 0.33 |
| CB-114 | 0.79±0.008 | 1.1±0.015 | 0.26±0.002 | 0.13 |
| CB-182 | 0.38±0.005 | 0.51±0.005 | 0.185±0.0005 | 0.06 |
| CB-126 | 0.38±0.006 | 0.51±0.004 | 0.185±0.0006 | 0.06 |
| CB-118 | 0.216±0.004 | 0.25±0.0002 | 0.154±0.0002 | 0.04 |
| CB-199 | 0.351±0.005 | 0.45±0.0026 | 0.18±0.0004 | 0.06 |
| CB-170 | 0.28±0.003 | 0.36±0.0025 | 0.168±0.0004 | 0.05 |
| CB-195 | 0.298±0.005 | 0.37±0.004 | 0.171±0.0005 | 0.05 |

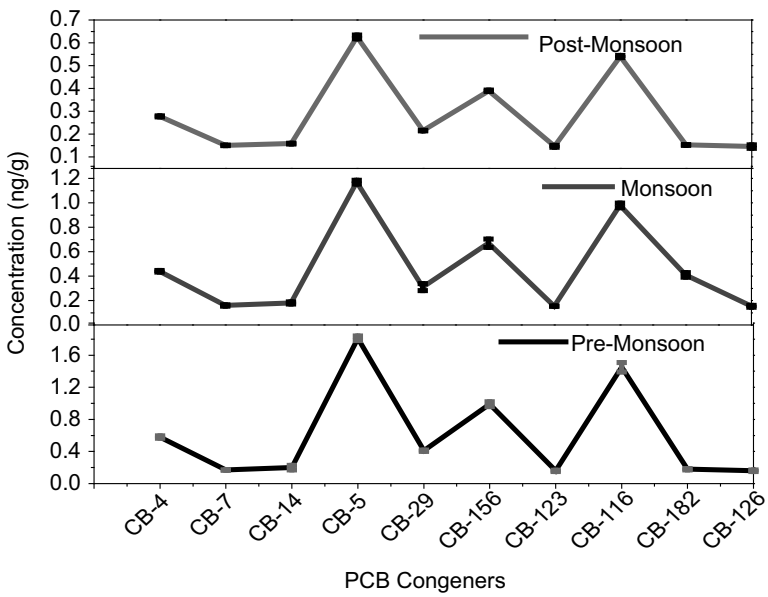


*Figure 3.3* Variation of PCB congeners at site-1 (Bargidhi Ghat) during various seasons.

sampling site. Table 3.4 and Figure 3.4 show the distribution pattern of various congeners of PCBs in the sediments of Subarnarekha River at the Adityapur Bridge site. The concentration levels of various congeners of PCBs at this site show the predominance of PCB-156 (0.76 ng/g with 39.92%) followed by PCB-11 (0.65 ng/g with 33.354%) and PCB-123 (0.28 ng/g, 2.189% composition).

*Table 3.4* Average seasonal variation in concentration of various congeners at the sampling site 3.

| *PCB Congeners* | *Average Concentration (ng/g) with Standard Deviation* | | | *% Concentration (ng/g)* |
|---|---|---|---|---|
| | *Pre-Monsoon* | *Monsoon* | *Post-Monsoon* | |
| CB-4 | 0.174±0.003 | 0.89±0.012 | 0.313±0.002 | 2.56 |
| CB-14 | 0.158±0.002 | 0.55±0.006 | 0.226±0.001 | 1.73 |
| CB-5 | 0.218±0.005 | 1.84±0.027 | 0.815±0.005 | 4.79 |
| CB-11 | 0.65±0.008 | 11.64±0.180 | 2.79±0.031 | 27.99 |
| CB-29 | 0.156±0.003 | 0.5±0.005 | 0.212±0.0008 | 1.61 |
| CB-81 | 0.22±0.002 | 0.55±0.006 | 0.235±0.0008 | 1.87 |
| CB-49 | 0.216±0.006 | 1.87±0.026 | 0.81±0.00500 | 4.84 |
| CB-156 | 0.76±0.010 | 14.37±0.210 | 3.54±0.03700 | 34.65 |
| CB-123 | 0.28±0.0005 | 0.364±0.003 | 0.191±0.0006 | 1.55 |
| CB-116 | 0.28±0.006 | 3.26±0.050 | 0.92±0.00900 | 8.28 |
| CB-157 | 0.141±0.0006 | 0.161±0.0004 | 0.146±0.0001 | 0.83 |
| CB-114 | 0.142±0.0008 | 0.164±0.0005 | 0.145±0.0001 | 0.84 |
| CB-153 | 0.141±0.0006 | 0.68±0.0008 | 0.152±0.0003 | 1.81 |
| CB-105 | 0.141±0.0005 | 0.162±0.0006 | 0.145±0.0002 | 0.83 |
| CB-138 | 0.147±0.0006 | 0.175±0.0005 | 0.148±0.0004 | 0.87 |
| CB-182 | 0.143±0.0008 | 0.305±0.003 | 0.174±0.0005 | 1.15 |
| CB-126 | 0.148±0.0007 | 0.217±0.0002 | 0.159±0.0002 | 0.97 |
| CB-185 | 0.146±0.0008 | 0.158±0.003 | 0.185±0.0002 | 0.91 |
| CB-118 | 0.146±0.0005 | 0.153±0.0002 | 0.143±0.0001 | 0.82 |
| CB-169 | 0.142±0.0006 | 0.153±0.0002 | 0±0.0000000 | 0.55 |
| CB-199 | 0.148±0.0005 | 0.156±0.0002 | 0±0.0000000 | 0.56 |

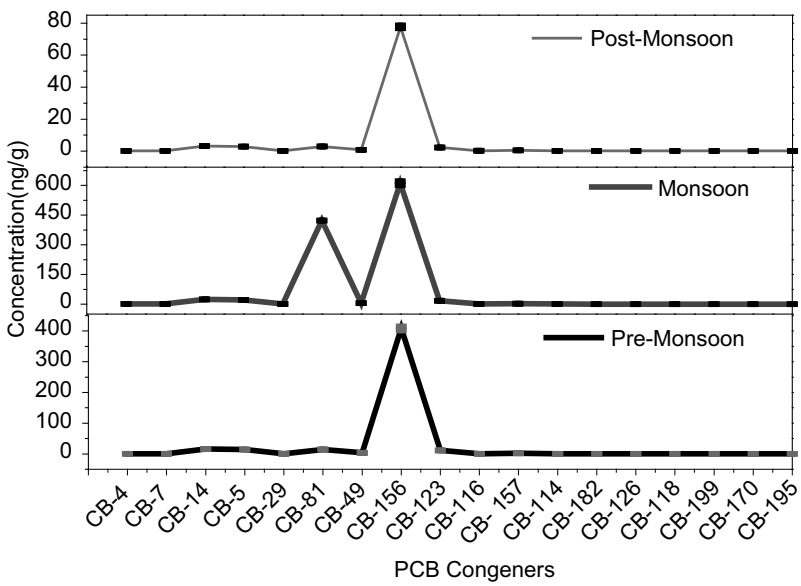


*Figure 3.4* Variation of $PCB_s$ congeners at site-2 (Mango Bridge) during various seasons.

### 5.4 *Mango Basti sampling site (Site 4)*

Total PCBs detected at the Mango Basti site during the pre-monsoon season was 6.34 ng/g. In this particular site, a total 11 congeners were detected. Out of the total congeners, seven were of co-planar type, namely PCB-5, PCB-29, PCB-123, PCB-114, PCB-105, and PCB-126, while the congeners like PCB-81, PCB-49, PCB-156, PCB-116, and PCB-182 were of non-planar type (Table 3.5 and Figure 3.5). The most predominant congeners of PCB were

*Table 3.5* Average seasonal variation in concentration of various congeners at the sampling site 4.

| *PCB Congeners* | *Average Concentration (ng/g) with Standard Deviation* | | | *% Concentration ng/g* |
|---|---|---|---|---|
| | *Pre-Monsoon* | *Monsoon* | *Post-Monsoon* | |
| CB-5 | 1.09±0.0150 | 1.13±0.0150 | 0.44±0.00400 | 13.70 |
| CB-29 | 0.26±0.0050 | 0.27±0.0002 | 0.184±0.0005 | 3.68 |
| CB-81 | 0.25±0.0060 | 0.26±0.0002 | 0.17±0.00050 | 3.50 |
| CB-49 | 0.3±0.00500 | 0.32±0.0003 | 0.205±0.0007 | 4.25 |
| CB-156 | 1.34±0.0500 | 1.54±0.0210 | 0.59±0.00500 | 17.87 |
| CB-123 | 0.25±0.0080 | 0.26±0.0020 | 0.17±0.00040 | 3.50 |
| CB-116 | 3.44±0.0500 | 3.63±0.0560 | 1.29±0.01400 | 43.04 |
| CB-114 | 0.18±0.0050 | 0.155±0.0002 | 0.146±0.00010 | 2.48 |
| CB-105 | 0.158±0.003 | 0.16±0.0003 | 0.148±0.00010 | 2.40 |
| CB-182 | 0.165±0.002 | 0.168±0.0004 | 0.149±0.00030 | 2.48 |
| CB-126 | 0.3±0.01500 | 0.158±0.0003 | 0.146±0.00020 | 3.11 |

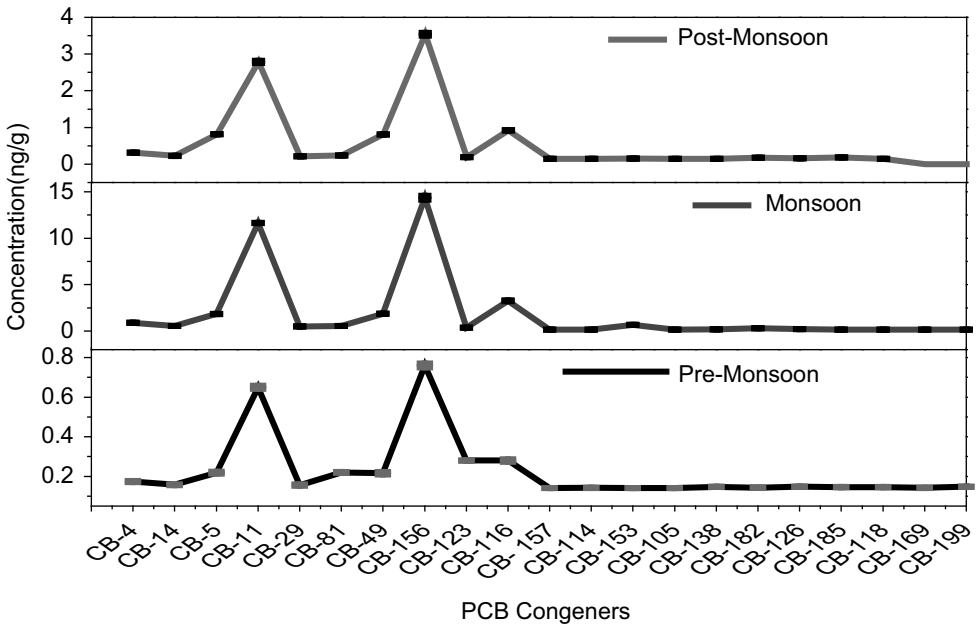


*Figure 3.5* Variation of PCB congeners at site-3 (Adityapur Bridge) during various seasons.

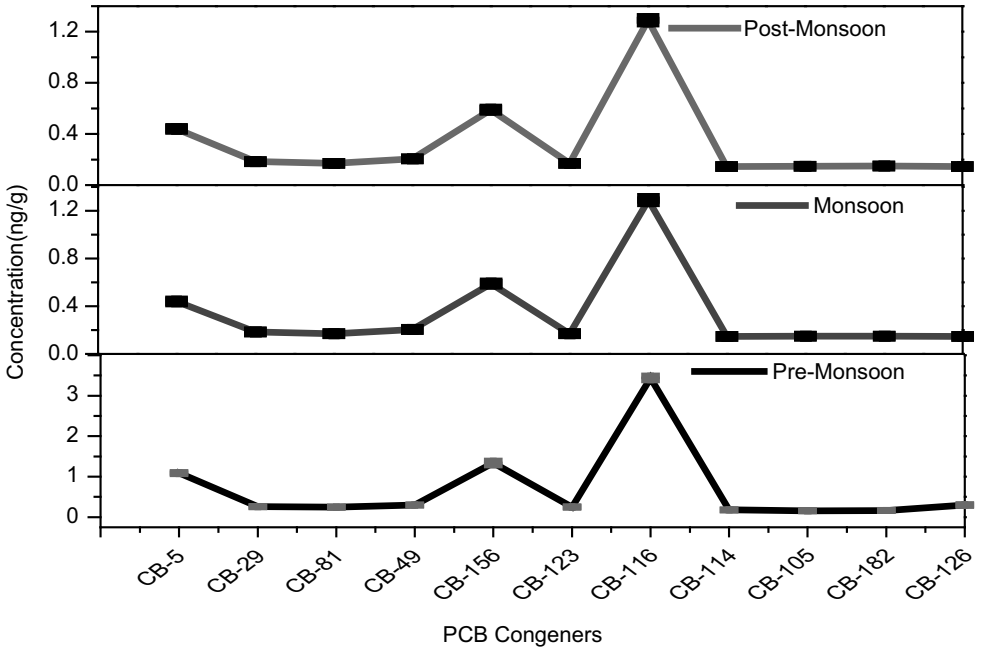


*Figure 3.6a* Variation of PCB congeners at site-4 (Mango Basti) during various seasons.

found to be PCB-116 (2,3,4,5,6-pentachlorobiphenyl) with a concentration of 3.44 ng/g constituting 53.778% of total PCBs followed by PCB-156 (2,2',4,5,5'-pentachlorobiphenyl) with a concentration of 1.34 ng/g and 20.190%, and PCB-5 (2,3'- dichlorobiphenyl) has a concentration of 1.09 ng/g having 14.35%.

### 5.5 *Adityapur Colony sampling site (Site 5)*

The sediment samples collected from Adityapur Colony sampling site showed a wide range of PCB concentrations. The lowest concentration of PCB congeners was found to be 0.154 ng/g (PCB-181), while the highest concentration was 10.50 ng/g (PCB-156). Table 3.6 and Figure 3.6 show the relative contribution of various congeners of PCB in the sediment of Subarnarekha River at Adityapur Colony. The most predominant congener was PCB-156 (2,2',4,5,5'-pentachlorobiphenyl) with a concentration of 10.50 ng/g (34% of the total PCBs). The total concentration of PCBs was estimated to be 25.94 ng/g in sediment during the pre-monsoon. Nineteen PCB congeners were detected in samples of Adityapur Colony. Among these, PCB-4, PCB-81, PCB-49, PCB-156, PCB-116, PCB-157, PCB-153, PCB-182, PCB-185, and PCB-181 belonged to non-planar type of PCBs, whereas, PCB-7, PCB-14, PCB-5, PCB-11, PCB-29, PCB-123, PCB-114, PCB-105, and PCB-169 possessed co-planar configuration. An overall assessment of the levels of contamination of different congeners of PCBs during the pre-monsoon indicates that all the 25 congeners were not detected at all the five sites as is evident from Table 3.2–3.6. It may be noted that, the congeners like PCB-29, PCB-123, PCB-116, and PCB-182, detected at the sampling site.

*Table 3.6* Average seasonal variation in concentration of various congeners at the sampling site 5.

| *PCB Congeners* | *Average Concentration (ng/g) with Standard Deviation* | | | *% Concentration ng/g* |
|---|---|---|---|---|
| | *Pre-Monsoon* | *Monsoon* | *Post-Monsoon* | |
| CB-4 | 1.2±0.01500 | 1.22±0.016 | 0.143±0.003 | 3.67 |
| CB-7 | 0.28±0.0080 | 0.28±0.002 | 0.167±0.0003 | 1.04 |
| CB-14 | 0.61±0.0050 | 0.61±0.006 | 0.23±0.0010 | 2.08 |
| CB-5 | 1.84±0.0150 | 1.94±0.023 | 0.5±0.0050 | 6.13 |
| CB-11 | 5.34±0.0500 | 5.44±0.080 | 2.04±0.0130 | 18.37 |
| CB-29 | 0.33±0.0250 | 0.33±0.003 | 0.18±0.0005 | 1.20 |
| CB-81 | 3.64±0.0330 | 0.53±0.0006 | 0.22±0.0012 | 6.29 |
| CB-49 | 0.59±0.0350 | 0.61±0.006 | 0.3±0.00130 | 2.15 |
| CB-156 | 10.5±0.8500 | 10.52±0.160 | 2.25±0.0250 | 33.34 |
| CB-123 | 0.23±0.0080 | 0.23±0.002 | 0.162±0.0003 | 0.89 |
| CB-116 | 5.44±0.0650 | 5.54±0.064 | 1.38±0.0130 | 17.71 |
| CB-157 | 0.17±0.0050 | 0.2±0.0004 | 0.147±0.0001 | 0.74 |
| CB-114 | 0.62±0.0080 | 0.18±0.0006 | 0.15±0.0001 | 1.36 |
| CB-153 | 0.159±0.0006 | 0.33±0.0002 | 0.144±0.0001 | 0.91 |
| CB-105 | 0.203±0.0030 | 0.2±0.0008 | 0.153±0.0002 | 0.80 |
| CB-182 | 0.169±0.0005 | 0.16±0.0003 | 0.146±0.0001 | 0.68 |
| CB-185 | 0.37±0.00300 | 0.37±0.0030 | 0.188±0.0005 | 1.33 |
| CB-181 | 0.154±0.0005 | 0.154±0.0002 | 0.143±0.0001 | 0.65 |
| CB-169 | 0.159±0.0006 | 0.159±0.0003 | 0.144±0.0001 | 0.66 |

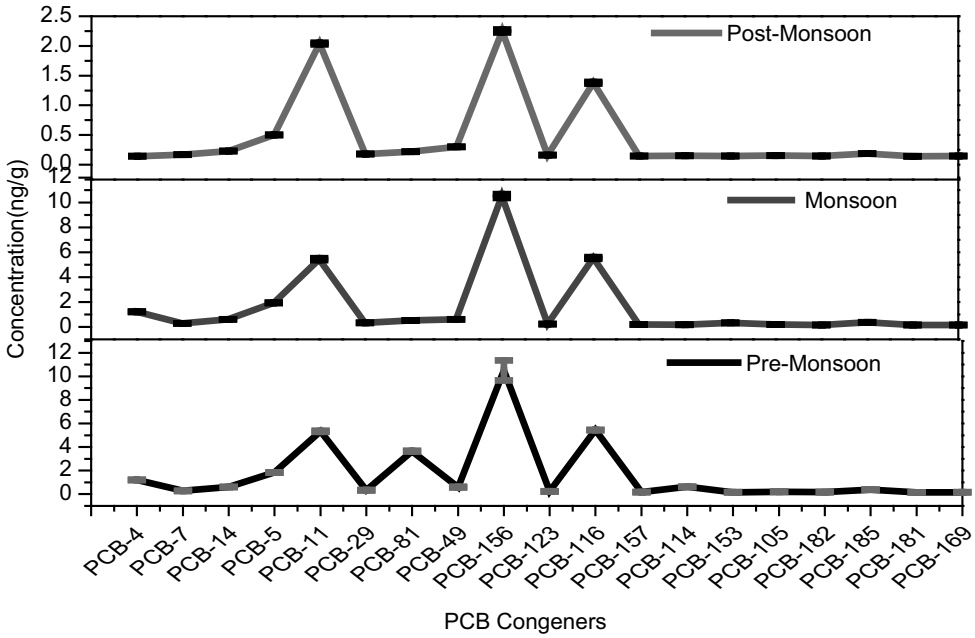


*Figure 3.6b* Variation of PCB congeners at Site-5 (Adityapur Colony) during various seasons.

## 6 Levels of contamination during the monsoon

### 6.1 *Bargidhi Ghat sampling site (Site 1)*

Table 3.2 shows the analysis of the variations in concentrations of PCBs in sediments at the Bargidhi Ghat sampling site during the monsoon period. Ten congeners of PCBs with a total concentration of 3.37 ng/g were detected at the Bargidhi Ghat sampling site during the monsoon season. The concentration of PCBs ranged from 0.15 ng/g to 1.17ng/g. Among the various congeners of PCBs detected the PCB-5 (2, 3-Dichlorobiphenyl) was the most predominant component with a concentration having 31.85% of total $PCB_s$.

### 6.2 *Mango Bridge sampling site (Site 2)*

The various congeners of PCBs detected in the sediments at Mango Bridge during the monsoon are: PCB-4, PCB-7, PCB-5, PCB-14, PCB-29, PCB-81, PCB-49, PCB-156, $PCB_s$-114, PCB-123, PCB-116, PCB-157, PCB-182, PCB-126, PCB-118, PCB-199, PCB-170, and PCB-195. Among these, the predominant congener was found to be PCB-156 (2,2',4,5,5'-pentachlorobiphenyl) with a concentration of 610.59 ng/g (85.92% of total PCBs). The concentration of total PCBs at the sampling site was found to be quite high (710.33 ng/g) during the monsoon. The concentrations of various congeners of PCBs were in the range of 0.25 ng/g to 610.59 ng/g.

### 6.3 *Adityapur Bridge sampling site (Site 3)*

Table 3.4 shows the variation in concentrations of different congeners of PCBs in sediment samples at the Adityapur Bridge site during the monsoon season. The predominant congener was found to be PCB-156 (2,2',4,5,5'-pentachlorobiphenyl), with a concentration of 14.37 ng/g, which constitutes 40% of the total PCBs. The concentrations of PCBs detected in the samples of Adityapur Bridge were in the range from 0.153 to 14.37 ng/g. The maximum numbers (21) of congeners of PCBs with a total concentration of 35.54 ng/g were detected at the Adityapur Bridge site during the monsoon season. The lowest concentration of PCBs estimated was 0.153 ng/g for PCB-169. It may be noted that PCB-4, PCB-81, PCB-49, PCB-156, PCB-116, PCB-157, PCB-153, PCB-138, PCB-182, PCB-185, and PCB-199 were of non-planar configuration because of the steric hindrance at the ortho-position of the biphenyl rings. On the other hand, PCB congeners like PCB-14, PCB-5, PCB-11, PCB-29, PCB-123, PCB-114, PCB-105, PCB-126, PCB-118, and PCB-169 were of co-planar configuration. The most toxic congeners of PCBs such as PCB-126 and PCB-169 were present in considerable amount in the sampling site, causing a serious concern of environmental pollution.

### 6.4 *Mango Basti sampling site (Site 4)*

Table 3.5 shows the variation in concentrations of various congeners of PCBs in sediments at Mango Basti during the monsoons. Eleven congeners of PCBs were detected at this site. Among these, PCB-116 (2,3,4,5,6-pentachlorobiphenyl) was the most predominant congener, with a concentration of 3.63 ng/g that constituted 55% of the total PCBs. The concentration of total PCBs was 6.93 ng/g. The lowest concentration was 0.16 ng/g for PCB-114.

### 6.5 *Adityapur Colony sampling site (Site 5)*

Table 3.6 shows the concentrations of various congeners in sediments at the Adityapur colony site. The most predominant congener found at the Adityapur Colony site during the monsoon period was PCB-156 (2,2',4,5,5'-pentachlorobiphenyl) with a concentration of 10.81 ng/g, which constituted 39% of the total PCBs. The concentration of PCBs was in the range of 0.154 (PCB-181) to 10.81 ng/g.

Overall, 25 congeners of PCBs were detected at different sampling sites during the monsoon. While the congeners like PCB-4, PCB-7, PCB-14, PCB-5, PCB-29, PCB-156, PCB-123, PCB-116, PCB-182, PCB-14, PCB-5, PCB-29, PCB-1 01, PCB-123, PCB-116, PCB-182, and PCB-126 were found to be present in considerable amount at the Bargidhi Ghat sampling site, some of the PCBs were not detected at the Bargidhi Ghat site. A total of eighteen PCB congeners were detected at the Mango Bridge sampling site. Interestingly, PCBs like PCB-14, PCB-153, PCB-105, PCB-138, PCB-185, PCB-181, and PCB-169 were found to be absent at Mango Bridge, although they were detected in the other sampling sites like Bargidhi Ghat, Mango Basti, Adityapur Bridge, and Adityapur Colony. On the other hand, some of the congeners like PCB-7, PCB-181, PCB-170, and PCB-195 were absent at the Adityapur Bridge site while PCB-138, PCB-126, PCB-118, PCB-199, PCB-170, and PCB-195 could not be detected at the Adityapur Colony sampling site during the monsoon season, although they were present in other sampling sites.

Such a variation in the distribution of various congeners of PCBs could be attributed to the type of waste materials being discharged at various sampling sites as well as the physico-chemical characteristics of the sediments, which exhibit variations in concentrations of various congeners of PCBs at five different locations of the Subarnarekha River during the monsoon. It clearly shows the predominance of congeners like PCB-5, PCB-11, PCB-81, PCB-99, PCB-156, PCB-114, PCB-182, PCB-181, PCB-118, PCB-199, PCB-170, and PCB-195 at the Mango Bridge site, substantiating the high levels of concentration in that region during the monsoon. The high concentrations of PCBs at the Mango Bridge samples could be due to the large input of contaminants from different discharge points around the Jamshedpur suburban areas as well as from agricultural run-off.

## 7 Levels of contamination during the post-monsoon

### 7.1 *Bargidhi Ghat sampling site (Site 1)*

Table 3.2 illustrates the variation in concentration of PCB congeners detected at the Bargidhi Ghat sampling site. The predominant congener found in this site was PCB-5 (2, 3-dichlorobiphenyl), with a concentration of 0.62 ng/g (35% of the total PCBs).

### 7.2 *Mango Bridge sampling site (Site 2)*

Table 3.3 shows the relative contribution of various congeners of PCBs at the Mango Bridge sampling site. The concentrations of different PCB congeners were in the range of 0.154 ng/g to 77.68 ng/g. The percentage composition of various congeners of PCBs indicates that PCB-156 was the most predominant congener at the sampling site with 86% of total PCBs.

### 7.3 *Adityapur Bridge sampling site (Site 3)*

The analysis of sediment from this location indicated the presence of as many as 19 congeners of PCBs, of which PCB-156 and PCB-11 were the most predominant contaminants (Table 3.4). The most toxic co-planar congener, PCB-126, was present in very low concentration (0.15 ng/g) constituting only 0.2% of the total PCBs present in that location. The concentrations of different congeners of PCBs were in the range of 0.0 to 3.54 ng/g.

### 7.4 *Mango Basti sampling site (Site 4)*

The variations in the concentration of PCBs detected at the Mango Basti site are shown in Table 3.5. The total concentration of the PCBs was found to be 2.29 ng/g with a range varying from 0.17 to 1.29 ng/g. The most predominant congener of PCBs was found to be PCB-116 (2,3,4,5,6-pentachlorobiphenyl) with a concentration of 1.29 ng/g (56% of the total PCBs). The structural configurations of various congeners of PCBs are of immense significance because of their relative toxic potential.

### 7.5 *Adityapur Colony sampling site (Site 5)*

The sediment samples collected from Adityapur Colony site showed the presence of the following PCB congeners and their concentrations listed in Table 3.6. The concentration of total PCBs detected at the sampling site was 5.44 ng/g. The concentration of different PCB congeners were in the range of 0.143–2.25 ng/g. The most dominant congener of PCBs was found to be PCB-156 (2, 2', 4, 5, 5' -pentachloro-biphenyl) with a concentration of 2.25 ng/g (39% of total PCBs).

All the 25 congeners of PCBs could not be detected at all the sites. For instance, PCB-11, PCB-81, PCB-49, PCB-157, PCB-114, PCB-153, PCB-105, PCB-138, PCB-185, PCB-181, PCB-118, PCB-169, PCB-199, PCB-170, and PCB-195 were not detected at the Bargidhi Ghat sampling site. Similarly, PCB congeners like PCB-14, PCB-153, PCB-105, PCB-138, PCB-185, PCB-181, and PCB-169 could not be detected at the most contaminated sampling site at Mango Bridge.

The PCB-7, PCB-181, PCB-178, and PCB-195 congeners were absent at Adityapur Bridge sampling site. At Mango Basti, PCB congeners like PCB-4, PCB-7, PCB-14, PCB-11, PCB-157, PCB-153, PCB-138, PCB-185, PCB-181, PCB-118, PCB-199, PCB-170, and PCB-195 were not detected at all and at Adityapur colony site PCB-138, PCB-126, PCB-118, PCB-199, PCB-170, and PCB-195 were not found. Such a variation in relative distribution and occurrence of PCBs at various locations can be expected to be greatly influenced by the amount of waste materials discharged through different outlets as well as the seasonal variation, especially during the monsoon.

The persistence of these PCBs in sediment was closely related to the physico-chemical characteristics (Table 3.1) of the ambient environment. The low chlorinated PCBs like PCB-5, PCB-11, PCB-81, and PCB-49 were found to be dominant in sediment from the Mango Bridge site (Site 2). Such a predominance of lower congeners of PCBs could be due to the degradation of higher congeners of PCBs under the prevailing abiotic and biotic conditions of the sediment. All the sampling sites, except Bargidhi Ghat were found to have high levels of PCB contamination, the most significant of which being the

Mango Bridge drain. This could be an indication that the input of pollutants from the industrial discharges was much higher than agricultural run-off at this site. The Mango Bridge drain, being the biggest drain in Jamshedpur, receives maximum pollutants due to the discharge of waste materials from industrial, agricultural run-off, and municipal sewage.

The fact that this area is contaminated with some co-planar PCB congeners (PCB-116, PCB-114, etc.) is a matter of great concern because of their toxic potentials in comparison with the corresponding dibenzodioxin and dibenzofuran. Another toxic congener PCB-156 was detected in significant amounts in sediment from the Mango Bridge drain, Adityapur Bridge, and Adityapur Colony. Results of the three seasons, namely, pre-monsoon, monsoon, and post-monsoon, show a wide range of concentrations of 25 congeners of PCBs at different sampling sites. Among the three seasons, the concentration of total PCBs was highest in the monsoon season (782.70 ng/g), followed by pre-monsoon (519.10 ng/g) and post-monsoon (107.90 ng/g) seasons. Such high concentrations of PCBs in sediments of the Subarnarekha River clearly indicate the use pattern of various synthetic chemicals, technical grades of PCBs by different industries, and the discharge of waste materials without proper treatment in the river. The sources of PCB also include volatilization from landfills and other PCB-wastes, spills, dredge spoils sewage sludge, and improper dumping to open areas. Besides, pollution may also occur during the incineration of municipal and industrial wastes. Most municipal incinerators are not effective in destroying PCBs. Explosions of transformers and capacitors may release significant amounts of PCBs into the environment. Similarly, the detection of several PCB congeners at the Mango Bridge, Adityapur Bridge, and Adityapur Colony indicates industrial discharges and the release of effluents at these sites and also accumulation through run-off, leaching, rain wash, etc.

## 8 Conclusion

The study on PCBs and pesticides provides an insight into the levels of contamination of the Subarnarekha River during different seasons. Although the contaminants were found in ppb levels, due to their persistence, bio magnification, and toxicity, their presence in the environment is a matter of great concern. Hence, it is suggested that appropriate measures should be taken to protect the Subarnarekha River waters from such contamination and an integrated effort should be made to prevent further contamination.

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Chapter 4

# Ecological threats of dioxin in soil

*Gulnaz T. Javan, Cigdem Yangin-Gomec and Sheree J. Finley*

## 1 Introduction

Dioxin compounds are among the most toxic and carcinogenic environmental micropollutants, and they are ubiquitous in the biosphere. These toxic compounds are unintentional by-products from anthropogenic emissions formed during a variety of highly exothermic industrial processes. The production of chlorine gas, incineration of materials, wood pulp bleaching to make white paper products, and even residential burning of trash releases dioxin into the atmosphere (Nording *et al*., 2006). Dioxins may also be produced due to chemical reactions that occur naturally in the environment, such as in forest fires, volcanic activity, and fresh, uncontaminated garden compost piles. Exposure to dioxin molecules is implicated in several health conditions, such as liver damage, heart and circulation problems, severe acne-like eruptions, reproductive toxicity in males and females, suppression of immune responses, and cancer (Bertazzi *et al*., 2001). The earliest evidence of undesirable environmental dioxin pollutants dates back to 1827 when emissions were released from a German chemical plant during the manufacture of sodium carbonate (Balzer *et al*., 2007). Other large land regions throughout the world have also been impacted by industrial and other anthropogenic activities that have emitted dioxins.

The term "dioxin" is a generic word for a group of structurally similar, polyphenol isomers that consists of eight different positions available for halogen bond substitutions. Over four hundred types of dioxin-related compounds have been proven; however, only 30 of these are measured to be dangerous (WHO, 1991). Dioxins and furans are a family of chlorinated aromatic compounds, polychlorinated dibenzo-p-dioxins (PCDD), and polychlorinated dibenzofurans (PCDF), respectively. Dioxins are classified as persistent organic pollutants (POPs) and these chemicals are among the most hydrophobic organic contaminants, which make them extremely toxic (Hanano *et al*., 2018). The most potent and toxic chemical is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Figure 4.1), which has been demonstrated to bind to thyroid hormone receptors and transduce persistent agonistic and/or antagonistic activity of uncontrollable catabolic responses (McKinney *et al*., 1985).

## 2 Interactions of dioxins in soil

Extensive studies of dioxins in ecosystems have demonstrated that these molecules interact extensively with soil, mud, sludge, and sediments in a variety of ways. Dioxin molecules are introduced into the soil through dry or wet deposition via aerial transport from

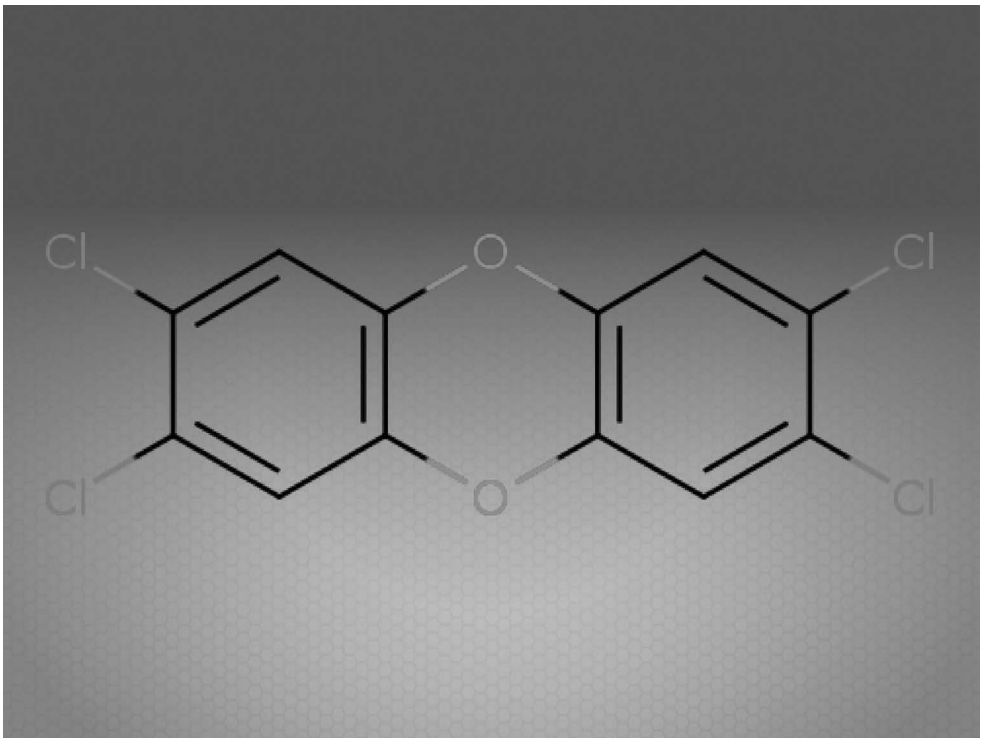


*Figure 4.1* Chemical structure of the most potent and toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

anthropogenic discharges and natural emissions (Weber *et al*., 2008). Dioxins present in the first three millimeters of soil disintegrate under ultraviolet rays in sunlight; however, deeper layers are not affected (Gibbs, 1997). Analogous to many other organic compounds, dioxins do not readily dissolve in water. Hence, many of these highly hydrophobic molecules can only enter water by strongly adhering to colloidal and dissolved organic matter (Dagouassat *et al*., 2012). Dioxins are then dispersed by a suspended solid phase and may travel extensive distances in water and eventually partition in topsoil, clay, loam, and mud. Their prevalence in soil leads to leaching through gradual vertical translocations into deeper subterranean layers of soils (Czerniak and Poszyler-Adamska, 2007). However, because of their strong adherence to hydrophobic particulate matter, dioxins do not reach groundwater; thus, they do not readily contaminate underground water flows.

The high physical, chemical, and biological stability of dioxins contribute to their persistence and bioavailability in soil. Dioxins are colorless, odorless solids with high melting and boiling points that are very resistant to evaporation in soil; therefore, they degrade extremely slowly. Thermal studies have shown that temperatures greater than 1400$^{o}$C are needed to completely disintegrate dioxin molecules (Lam *et al*., 2010). Natural characteristics, especially of the highly chlorinated dioxins (e.g., polychlorinated dibenzo-p-dioxins and dibenzofurans), such as low vapor pressure, low water solubility, and high octanol-water partitioning coefficient, make them have a high affinity to particles and

extremely slow evaporation and leaching rates from the soil. Moreover, percolation of water through soil does not readily eliminate lipophilic dioxins from soil particles. The bioavailability of dioxin is low, and hence its biodegradation is also very slow, providing long half-lives up to 15 years in surface soil, more than 25 years in subsurface soil, and up to 100 years in soil interiors and sediments (Paustenbach *et al*.,1992).

Dioxins bind very strongly to solid particles, soil colloids, and other organic matter by encapsulation into particle pores (Li *et al*., 2007). The binding of dioxin depends on the soil particle size; smaller soil particles have higher surface area-to-mass ratios than larger particles. Thus, generally, smaller soil particles (less than 250 μm) have higher concentrations of dioxins in soil (US EPA, 2002). After deposition on soil, contaminants are redistributed from weak adsorption sites to stronger, from the surface to the subsurface, and even further into the finer pores. When dioxins are adsorbed into soil particles, the chemical stability of these compounds unfortunately increases, which leads to these chemicals' resistance to most common acids, bases, oxidizing agents, and reducing agents at ambient temperatures as well as more difficulty in being degraded. Therefore, strong reducing, oxidizing, or photolytic conditions, and/or high temperatures, are generally required to degrade the dioxins from contaminated soils (Haglund, 2007). All the aforementioned inherent problems associated with these toxic compounds necessitate inexpensive and feasible remediation techniques for dioxin-polluted soils. In this context, bioremediation is considered a most effective method of dealing with widespread pollution.

## 3 Human exposure from dioxins in soil

Bioavailability studies have been performed to determine the routes of entry of dioxin from polluted soils into mammalian bloodstreams (Figure 4.2). In *in vitro* dermal uptake studies of TCDD from contaminated soil, it has been demonstrated that this particular congener inefficiently penetrates into the skin of adult rats (Banks and Birnbaum, 1991). The study revealed that the amount of administered doses detected in the rats' blood was less than 0.3%. Therefore, it was concluded that skin is not a viable vehicle of exposure that leads to biomagnification in mammalian blood (Banks and Birnbaum, 1991; Weber *et al*., 1991). No matter which way dioxin enters the human body, it passes into the bloodstream and remains in the circulatory system for a very short time because of its insolubility in water and affinity for fatty tissues. Biomagnification of dioxins in humans actually occurs in "secondary" exposure as molecules are transported from the soil into the food chain through consumption of contaminated vegetation and soil-based feed by commercial livestock (Turrio-Baldassarri *et al*., 2007).

In recent years, increased industrialization, along with by-products such as dioxins and many similar chemicals that are produced during the production of chlorinated products, has become a significant risk to human health and environmental assessments. Human exposure to dioxins has been related to a wide range of toxic effects, including immune system damage, endocrine disruption, developmental disabilities, and reproductive disturbances. Dioxin, by forming strong bonds with specific liver proteins such as plasma proteins, can remain in the liver and fatty tissues for many years (Kang *et al*., 2005). According to the United States Environmental Protection Agency (EPA), the half-life of dioxin in humans is between seven to 14 years (Pirkle *et al*., 1989). Vertebrates with more body fat can store larger amounts of dioxin in their bodies. In animals, as fats are

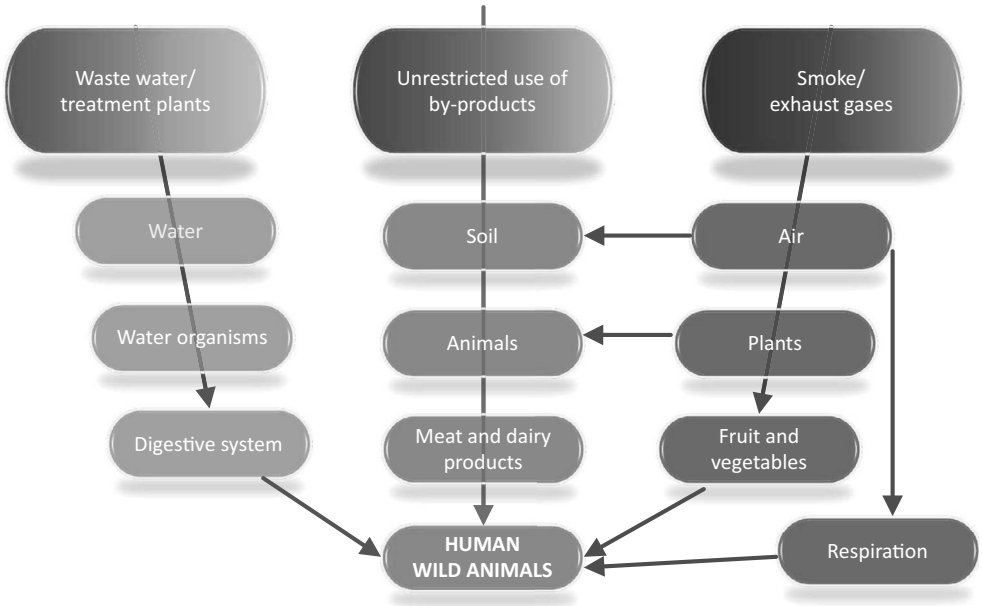


*Figure 4.2* Dioxin path to the food chain. Routes of entry of dioxins into the food chain leading to bioaccumulation in human and animal bloodstreams.

metabolized, the stored dioxins become mobilized and are excreted in human wastes and are redeposited on soil.

## 4 Dioxin concentrations in different geographic locations

The toxicological potency of dioxins in abiotic environmental samples such as soil, sediments, and water are measured in toxic equivalent (TEQ) (Urban *et al*., 2014). TEQ concentrations are quantitated according to the following mathematical equation:

$$TEQ = \Sigma_{n1}[PCDD_i \times TEF_i] + \Sigma_{n2}[PCDF_i \times TEF_i] + \Sigma_{n3}[PCB_i \times TEF_i] \quad (1)$$

TEF is toxic equivalency factor, and PCB is polychlorinated biphenyl. An extensive review of published TEQ data concerning background amounts of dioxin and dioxin-like compounds in soils in the United States determined that the levels were highly variable, 0.1–186 ng/kg TEQ in industrialized urban/suburban soils and 0.1–22.9 ng/kg TEQ in less-industrialized rural soils (Urban *et al*., 2014). In the United States, the TEQ quantities are commonly used as relative indicators of soil and sediment accumulation in order to prioritize remediation efforts (Table 4.1) (Van den Berg *et al*., 1998; US EPA, 2006).

Despite the fact that the formation of dioxins occurs as a result of local industrial and environmental processes, dispersal of these toxic molecules has extensive geographical distribution. Across the United States, studies have shown that there are approximately 500,000 tons of dioxin contamination in soil and sediments (Haglund, 2010). For

*Table 4.1* Sources of dioxin pollutants in soil

| *Source* | *1987* | | *1995* | | *2000* | |
|---|---|---|---|---|---|---|
| | *g TEQ* | *%* | *g TEQ* | *%* | *g TEQ* | *%* |
| Municipal wastewater sludge applied to soil | 85.0 | 0.6% | 133.3 | 3.9% | 89.7 | 6.3% |
| Ethylene chloride/vinyl chloride production | ND[a] | <0.01% | 35.7 | 1.0% | 30.0 | 2.1% |
| Bleached chemical wood pulp and paper mills | 370.1 | 2.7% | 30.0 | 0.9% | ND[a] | <0.01% |
| 2,4-Dichlorophenoxy acetic acid | 33.4 | 0.2% | ND[a] | <0.01% | ND[a] | <0.01% |

Quantifiable sources of dioxin pollutants in soil (g TEQ) and the percentage of dioxin in samples from all environmental sources (air, water, and soil) in 1987, 1995, and 2000, respectively.

[a] ND = Not detected in soil (US EPA, 2006).

example, dioxin deposition in sediment cores sampled from Siskiwit Lake near Lake Superior, USA between 1940 and 1970 revealed that the deposition rate increased eightfold during that period (Thomas and Spiro, 1996). Thereafter, the rate declined by approximately 30% due to improvements in the technology used in municipal incinerators and the decrease in forest fires. In another example, dioxin contaminants were detected in soil from the Love Canal suburb of Niagara Falls, New York, USA in 1978. Concentrations of TCDD were as high as 312 ng/g in sediment samples (Smith *et al*., 1983). Another environmental disaster that introduced elevated levels of dioxins into the soil happened between 1982 and 1985 in Times Beach, Missouri, USA. Several deaths of horses and illnesses of horse riders were attributed to high TCDD concentrations in soils (Fries, 1995). The TCDD was a component of oil used to control dust on unpaved streets in Times Beach. The entire town was evacuated and eventually all properties were bought by the US EPA, and the municipality was disincorporated. As a result of the exposure in Times Beach, a significant number of affected humans, particularly children, developed chloracne, that is, severe acne-like dermal disfigurations (Suskind, 1985).

Another environmental dioxin tragedy occurred involving Spolana Neratovice, a company in the Czech Republic that supplied the United States military during the Vietnam War with 2,4,5-trichlorophenoxyacetic acid, a chemical that contains trace amounts of the very toxic TCDD. In 1968, an explosion caused heavy contamination within the plant. Later in 2002, a flood in the local area spatially distributed large amounts of residual dioxins into the nearby Elbe and Mulde Rivers. The toxic chemical was then partitioned in the surrounding soils. Concentrations of dioxins were 12 pg/g TEQ at 173 km upstream of Mulde and 68 pg/g TEQ at 318 km downstream of Mulde (Umlauf *et al*., 2011). Similar to the Times Beach exposure, the most substantial manifestation of dioxin contamination in humans was the development of chloracne in children (Suskind, 1985).

The highest report of concentrations of dioxin emissions into the ambient environment were reported due to the occurrence of terrorist attacks on the World Trade Center in New York City, on September 11, 2001 (Tonev *et al*., 2009). The EPA Office of Research and Development in Washington, DC reported that dioxin levels in the Trade Center rubble ranged from 10 to 170 parts per trillion (ppt), which is approximately six times greater than the highest dioxin quantity recorded in the history of dioxin levels.

Land studies in Vietnam have also been performed to assess the contamination of dioxins used for defoliation of jungles by the United States military's herbicidal warfare program during the Vietnam War (Schecter *et al*., 1995; Dwernychuk *et al*., 2002). The herbicidal chemical spray used from 1961 to 1971 was a mixture of Agent Orange that

contained equal amounts of 2,4,5-trichlorophenoxyacetic acid, which contains trace amounts of the very toxic TCDD, and 2,4-dichlorophenoxyacetic acid. The United States military conducted approximately 20,000 aerial spray operations over Vietnam that released more than 350 kg of dioxin over the south and central areas of the country that covered more than 2.5 $x10^{10}$ $m^2$ (Stellman *et al*., 2003). Agent Orange was also used for deforestation of the perimeter regions encompassing military bases and unfortunately, was heavily spilled near the Bien Hoa military base (Dwernychuk *et al*., 2002). Heavily contaminated soil and sediments in Vietnam contain dioxin concentrations that ranged from 2.7 mg/kg dry weight in Can Gio, a region south of Vietnam, to 9.6 mg/kg dry weight in Hanoi, a northern region of Vietnam. Interestingly, three former military bases had higher levels of dioxin in soil compared to distant sites that received aerial spray (Dwernychuk *et al*., 2002). Concentrations as high as one million ppt of one of the most potent dioxins, TCDD, have been detected in soil and sediments in areas in Vietnam that were contaminated with Agent Orange (Tonev *et al*., 2009).

## 5 Dioxin concentrations in agriculture soils

Plant physiological studies have resulted in analogous conclusions that there are negligible amounts of dioxins that translocate from the agricultural soil through the plant vascular system to the edible above-ground parts of the plant (Kirchmann and Tengsved, 1991). In an extensive study involving rice plants grown in a patty field contaminated with approximately 120 pg/g TEQ of dioxin, the findings demonstrated that concentrations increased in the following order: grain, stems, husks, and leaves (Uegaki *et al*., 2006). Dioxin concentrations were very low in the grain, and undetectable in the xylem sap. In other words, amounts increased towards the outer parts of the plants primarily due to atmospheric gases in the environment as opposed to uptake of the toxin from the soil.

One reason that the toxin does not readily translocate is due to strong lipophilic binding and adherence to soils. Studies have demonstrated the uptake of dioxins occurs from soil to the roots only. However, root vegetables that ripen in the ground, such as turnips, potatoes, and carrots, may have residual amounts of dioxin-contaminated soil on their exteriors. For example, carrots grown in soil that was fertilized with sewage sludge had elevated concentrations of dioxins in the below-ground, lipid-rich exterior layers compared to control vegetables (Engwall and Hjelm, 2000). Small concentrations of dioxins in contaminated soil may increase, or bioaccumulate, up the food chain to hazardous amounts due to their resistance to metabolism, low water solubility, and long half-life (Tonev *et al*., 2009). Interestingly, much of the dioxin contamination has been introduced into the food chain in the United States via naturally, dioxin-contaminated ball clay, a binder and anti-caking agent added in different agricultural feed (Hens *et al*., 2016). After this finding, the use of ball clay as an animal food additive was prohibited by the Food and Drug Administration (FDA).

## 6 Mitigation of threats from dioxin-contaminated soils

The remediation of dioxin-contaminated sites is one of the most challenging tasks in environmental technology due to the fact that these dioxins cling persistently to soil particles. Hundreds of thousands of metric tons of dioxin-contaminated soil and sediments are reported in the United States alone, and hence, there is a great requirement for effective

remediation techniques that could be applied to clean the polluted areas and provide safe usage of these sites. However, most of the remediation methods (e.g., the use of ultraviolet light, chemical reagents such as hydrogen peroxide and super critical water) are very expensive, which makes their applications to large areas unfeasible. Indeed, the amount of dioxins discharged into the environment should be reduced initially by measures that decrease dioxin levels in discharge from incinerators. However, cost-efficient methods are still needed to be applied for the mitigation of threats from dioxin-contaminated soils (Haglund, 2007; Zoller and Ballschmiter, 1986).

In recent years, by the isolation and characterization of large numbers of microorganisms that are capable of degrading dioxins and dioxin-like compounds, it has become clear that biological methods using these particular microorganisms and microbial consortia have greater appeal than physicochemical methods in their application for environmental remediation. The biodiversity, ecophysiology, and molecular biology of dioxin-degrading microorganisms have been investigated extensively during the past decade (Hiraishi, 2003). Hanano *et al*. (2018) reported that identification of dioxin-degrading bacteria from contaminated sites should allow bioremediation to be carried out (Hanano *et al*., 2018). Besides, it was also reported that the selection of new bacterial species with potential dioxin removal activities could be better done by measuring some microbiota biological parameters (density, species diversity and richness, and genetic profiling) in severely dioxin-polluted soils.

There are three major modes of microbial degradation and transformation of dioxins: (i) oxidative degradation by aerobic bacteria containing aromatic hydrocarbon dioxygenases, (ii) reductive dechlorination by anaerobic microorganisms, and (iii) fungal oxidation with specific enzymes (e.g., cytochrome P450, lignin peroxidases and laccases) (Hiraishi, 2003).

In a related study, a screening method was described for fungi that could degrade dioxins and the biodegradation of three kinds of dioxins in soil with fungi (Tachibana *et al*., 2007). It was reported that the rate of degradation increased as the amount of fungus added increased. Also, it was indicated that more than 60% of dioxins in contaminated soil were degraded by bioremediation over 30 days, and the maximal rate of degradation (90%) was obtained when the bioremediation was conducted for 30 days in the presence of 0.1% surfactant.

In another study by Chen and Wang (2013), the results showed that high levels of near-fully and fully chlorinated dioxins (i.e., particularly octachlorodibenzofuran) were effectually reduced without accumulation of less-substituted congeners (Chen and Wang, 2013). The clone library analysis of PCR-amplified 16S rRNA genes from the octachlorodibenzofuran-degrading consortia showed that approximately 98% of the detected sequences were affiliated with *Proteobacteria*.

Although biodegradation is the cheapest method for the destruction of dioxins among all the methods described, efficient hybrid organisms have to be constructed in the laboratory for the maximum destruction of these compounds (Kulkarni *et al*., 2008). Chen and Wang, (2013) also reported that a distinctive microbial composition and population dynamic could be required for the enhanced degradation of highly chlorinated dioxins and furans in the batch microcosm and highlight a potential of bioremediation technologies in remedying these toxic compounds in polluted sites (Chen and Wang, 2013).

Many countries have assessed the toxicity of dioxins in soil and have established acceptable concentration levels, which are to provide protection to humans. WHO published, for the first time, evaluations of the worldwide commitment of food borne disease in 2015.

Decreasing exposure to dioxins is a major public health goal for disease reduction. To achieve this, WHO organized several meetings to find ways to determine human dioxin intake. At the conclusion of these meetings, a provisional tolerably monthly intake (PTMI) of 70 pg/kg was established as the acceptable amount of dioxin per month (WHO).

## 7 Effects of dioxin exposure on industry workers, children, and pregnancy

The history of anthropogenic dioxin production and dioxin poisoning is more than 200 years old (White and Birnbaum, 2009). Epidemiologic data on human exposures have revealed profound consequences, especially when humans are exposed to dioxins early in life, with respect to several immunologic, neurologic, and reproductive developmental endpoints. For example, after many decades of extensive dioxin soil contamination, chloracne, a persistent cystic and hyperkeratotic skin condition, was identified in German industrial workers. Chloracne is an acne-like eruption of blackheads, cysts, and pustules, which is related to over-exposure to certain halogenated aromatic compounds (e.g., chlorinated dioxins and dibenzofurans) (Suskind, 1985). An explosion in a Monsanto chemical plant in Nitro, West Virginia occurred in 1949. It was reported that the exposure of workers to the dioxin-contaminated herbicide, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) caused persistent chloracne after this incident. Moreover, an increase was observed in all cancers combined, particularly among the most highly and the longest dioxin-exposed workers occupationally (Suskind, 1985; Fingerhut *et al*., 1991; Suskind and Hertzberg, 1984).

On the other hand, there are huge differences in uptake rates between humans of different ages. It was reported that infants and children absorb higher rates of dioxins than adults because even at small doses, dioxin-like chlorinated aromatic compounds are more readily and effectively absorbed (Gies *et al*., 2007). According to WHO (1998), the dose per unit body weight (bw) for children decreases with increasing age, but the daily dose (pg/day) increases with age and remains almost constant in adults over 20 years of age. In this context, human health risk assessments for dioxins and dioxin-like PCBs recommend health-based exposure limits within the range of 1–4 pg WHO-TEQ/kg bw per day where TEQ refers to total dioxin toxic equivalents.

Since children consume more food than adults in relation to their body weight, they ingest higher doses of dioxin-like compounds than adults. Previous studies reported that developmental delays were observed in children and the mean daily intake could be three times higher than in young adults in the groups of one- to ten-year-old children. In a related study by Patandin *et al*. (1999), a mean daily intake of these compounds in 20–25 years old Dutch adults was determined as 2.3 and 2.0 pg TEQ/kg bw per day for males and females, respectively (Patandin *et al*., 1999). Moreover, skin lesions, fatigue, and altered reproductive and immunologic function are the symptoms of an illness namely "Yusho" (literally, "oil") disease, which refers to a condition caused by specific mass poisoning with PCBs and PCDFs in rice bran oil, that occurred in northern Kyushu, Japan, in 1968 (Loganathan and Masunaga, 2015). Accordingly, women exposed to PCDDs, PCDFs, and PCBs after this rice oil contamination incident had higher rates of spontaneous abortion and pregnancy loss in about ten years after exposure (Tsukimori *et al*., 2008). Hence, studies in highly exposed populations with a wider range of exposure could help clarify the connection between TCDD exposure and pregnancy

outcomes (Wesselink *et al*., 2014). Moreover, novel findings indicated that exposure to TCDD during pregnancy disrupts mammary gland development, resulting in impaired lactation and decreased offspring survival (Vorderstrasse *et al*., 2004). Furthermore, Lorber and Phillips (2002) demonstrated that infants who breast-fed for one year had accumulated doses of dioxin-like compounds which were six times higher than a one-year-old infant who was formula-fed (Lorber and Phillips, 2002). A greater understanding of the mechanisms involved in dioxin exposure of infants due to lactation will ultimately help reduce the burden of disease on adult populations.

## 8 Preventative parameters to the general public

There is a very broad range of toxic effects of "dioxins." Because dioxins accumulate in the food chain, they can have serious effects on human health. Dioxins are found throughout the world in the environment and they accumulate in the food chain, mainly in the fatty tissue of animals. Hence, although the consumption of food contaminated with "dioxins" need not directly cause a toxic effect; repeated ingestion of contaminated food could result in an increase of the "body burden" and thus chronic toxicity, showing that the exposure of human to dioxins should be minimized wherever possible. Avoiding exposure is not easy because of its ongoing release by various industries, which has led to broad contamination of the food supply. More than 90% of human exposure is through animal products, primarily meat, including also fish and shellfish as well as dairy products (e.g., milk, eggs, butter, etc.). Hence, dioxin toxicity can be relatively avoided by eating fewer animal products and by cutting down the high exposure foods (Nau, 2006; WHO, 2016).

As all humans are exposed to measurable levels of dioxins and related substances, the determination of the tolerated daily intake is a very prominent decision and may affect limit values guiding risk reduction measures and target levels. The recent limit values recommended by international authorities such as WHO, JECFA (Joint Expert Committee on Food Additives), etc. for PCDDs, PCDFs and dioxin-like PCBs indicate that these values were derived only from non-carcinogenic endpoints. This is particularly important for infants who are exposed to these toxic chemicals through breast-feeding, because infants may exceed the exposure of adults by one or two orders of magnitude. Hence, the only way to protect a child pre- and post-natal against dioxin uptake is to drastically reduce the background exposure of the general population, an objective which should be included in the risk assessment. By including the vulnerable group of children into the risk assessment process, the whole human population would be protected against the toxic effects of dioxins. Moreover, prevention or reduction of human exposure is also best done via source-directed measures, such as strict control of industrial processes to reduce formation of dioxins (Gies *et al*., 2007; WHO, 2016; Carpenter and Bushkin-Bedient, 2013).

## 9 Conclusion

In conclusion, the goal of this chapter was to provide an in-depth review of dioxin interactions in soil in various geographic locations, as well as to present an overview of the effects of human exposure to dioxins and dioxin-related compounds. As stated throughout this chapter, dioxin molecules are discharged through natural occurrences and accidental

spillages into proximate environments and interact extensively with soil in a variety of ways. These compounds are among the most deadly and carcinogenic substances in the world. With strict regulatory controls on major industries around the world, sources of dioxin emissions have drastically declined in the environment. The large number of studies, proceedings, and regulations related to dioxin toxicity reflect the recognized value of the ecological threats of these chemicals in soil. Many scientists and governmental entities are currently elucidating the sources of dioxin emissions, their persistence in soil, and the possible effects on human health. The treatment of dioxin contamination in soil is expensive and is not a trivial task. Regulations for cleanup and proper disposal entail remediation treatments to reduce dioxin to very low residual levels. Currently, no technology can completely eliminate dioxin from soil. Moreover, some remediation technologies may pose other health threats, which make the general public skeptical of the proposed technologies.

Continued research initiatives to determine the amount of soil pollution in different terrestrial habitats throughout the world are needed to establish risk assessment and risk reduction measures for human health and the environment. The next step in dioxin research is to fully determine the cause and effect relationships of dioxin exposure, as well as the biological mechanisms and potential health hazards in humans. Assessments of ecological threats of dioxin in soil will necessitate consistent, interdisciplinary, and collaborative approaches among laboratories. Furthermore, future studies will undoubtedly create new discussions due to previously unreported discoveries of dioxin and dioxin-like contamination in soils and ecosystems. Future studies should also include longitudinal inquiries into the epigenetic effects of second- and third-generation toxicity of dioxin in mammalian bloodstreams that affects mammalian sexual differentiation and puberty development, mental cognition, behavior, and other adverse associations. Thereafter, new policies and regulations to safeguard the general public against dioxin exposure need to be formulated and implemented. Additionally, future studies will apply state-of-the art environmental remediation (e.g., bioremediation using genetically modified microorganisms) and determine legal obligations, liability compensation, and emergency response to dioxin exposure in soil.

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Chapter 5

# Dioxin in food

*Perugini Monia and Daniela Zezza*

## 1 Introduction

Dioxins are a group of chemical compounds of anthropogenic origin that include 75 polychlorinated dibenzo-p-dioxins (PCDD) and 135 polychlorinated dibenzofurans (PCDF) congeners. Dioxins are persistent environmental pollutants that tend to accumulate in the food chain. PCDD and PCDF congeners show a different degree of toxicity, depending on their highly toxic potential, but all congeners refer to the most toxic one – the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Several dioxin-related food incidents have raised considerable public concern in the recent years. Dioxins can enter the human body through breathing contaminated air, drinking contaminated water, or eating contaminated food. The central human intake is represented by food, and animal feed can make a significant contribution to the contamination of food. In 2002, the European Commission prescribed a list of actions to reduce the presence of dioxins and dioxin-like PCBs further and later introduced regular monitoring of food and feed by the Member States (EFSA, 2010).

## 2 Occurrence of dioxin

Dioxins are a group of chemically-interrelated compounds that are persistent environmental pollutants (Liem and van Zorge, 1995). There are 210 theoretically possible congeners divided into two groups: polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) congeners. Dioxins are persistent against chemical and microbiological degradation, and they are found throughout the world in the environment because they are very persistent. It is generally accepted that about 90–98% of human exposure to dioxins come from the dietary intake for non-occupationally exposed persons (De Mul *et al*., 2008). PCDD/Fs are produced naturally, such as during the forest fires (EPA, 2004), or anthropogenically through unregulated discharge from a variety of industrial processes as well as uncontrolled burning of household waste (Lemieux *et al*., 2003). Due to their persistence, they accumulate in the food chain, mainly in fatty tissues, and food of animal origin was shown to contribute significantly to human intake.

However, different dietary habits of subpopulations and various cultural, religious, and ethnic groups across the world lead to variations of the types of food groups implicated. A direct relationship between food contamination and feed contamination exists. This relationship occurs through the deposition of contaminated emissions on farmland, while fish accumulate dioxins through pollution of the aquatic environment. For this reason,

*Table 5.1* Selection of dioxin incidents and related contamination sources since 1999.

| *Year* | *Country* | *Product* | *Source* | *Reference* |
|---|---|---|---|---|
| 1999 | Belgium | Animal feed | Transformer oil | Larebeke *et al.* (2001) |
| 2003 | Germany | Animal feed | Use of waste wood for drying of the bakery waste | Hoogenboom *et al.* (2004) |
| 2004 | Italy | Eggs, meat | Wood-shaving litter | Diletti *et al.* (2005) |
| 2004 | Netherlands | Milk as a result of animal feed | Potato by products contaminated by kaolinitic clay | Hoogenboom *et al.* (2005) |
| 2006 | Netherlands | Pig feed | HCl containing dioxins and used in a gelatin production plant contaminated pig fat | Hoogenboom *et al.* (2007) |
| 2008 | India | Guar gum | Use of pentachlorophenol | Wahl *et al.* (2008) |
| 2008 | Italy | Mozzarella from buffalo milk | Milk, Illegal waste burning | Borrello *et al.* (2008) |
| 2008 | Ireland | Pork and beef | Bakery waste: PCB-contaminated fuel for drying of animal feed | Tlustos (2009) |
| 2008 | Korea | Pork | Dioxin contamination of Chilean pork from zinc oxide in feed | Kim *et al.* (2011) |
| 2008 | Netherlands | Feed additive | Brominated dioxins in feed additives | Traag *et al.* (2009) |
| 2011 | Germany | Animal feed | Use of contaminated residues from biodiesel production | Weber *et al.* (2011) |

animal feed is a large source of the contamination of food. Table 5.1 shows a selection of dioxin incidents and related sources since 1999, confirming the full range of possibilities of contamination of food and feed.

Dioxins are present in several foods, but the levels of contamination depend on the origin of the foodstuff. Many authors (Malisch and Kotz, 2014; De Mul *et al*., 2008; Charnley and Doull, 2005; Godliauskienė *et al*., 2012) reported levels of PCDD/Fs in food and feed, but the comparison among the results was not always possible because of how the results were expressed: on a fat basis, on whole weight basis, or for feed, on moisture basis. In the European Union, the maximum residue levels of PCDDs is expressed on a lipid basis for all foodstuffs, except fish and fish products. Considering the results presented in the SCOOP database (SCOOP, 2000), it is possible to see there are significant differences in the amount, detail, and quality of the data from the participating countries (Belgium, Denmark, Finland, France, Germany, Italy, Netherlands, Norway, Sweden, and the United Kingdom). In some cases, an upper-bound approach was used with the LOQ in calculating toxic equivalents for those congeners that were not quantified. In other cases, a lower-bound approach, with a zero value for those congeners that were not quantified, was used, underestimating the PCDD/Fs concentrations.

Furthermore, the results appeared to have been obtained mainly without adequate harmonization of analytical procedures and inter-calibration among the laboratories in different countries. This affects the comparability of the results, as also reported from Becher *et al*. (2004). To assure comprehensive, reliable data on the presence of dioxin-like compounds in food and feed, the EU Commission established requirements and quality criteria regarding the methods of analysis to be used in the official control of levels of dioxin-like compounds in foodstuffs (European Commission, 2002). Among others, the directive states that "… The continuous participation in inter laboratory studies for the determination of

dioxins and dioxin-like PCBs in the relevant feed/food matrices is mandatory." Several international interlaboratory comparison studies on dioxins and dioxin-like PCBs have been performed on biological matrices, such as cow's milk, fish, and breast milk (WHO/ECEH, 1995; De Boer and Wells, 1997; FAPAS, 2002).

Furthermore, in 2002 the European Commission prescribed a list of actions to reduce the presence of dioxins and dioxin-like PCBs further and later introduced regular monitoring of food and feed by the Member States (EFSA, 2010). Norway and Iceland and 19 Member States have taken part in this monitoring program where a total of 7270 samples were collected and analyzed in the period 1999–2008. These results, analyzed from EFSA (2010), reported that the highest average levels of dioxins and dioxin-like PCBs in fat content were observed for liver (5.7 pg TEQWHO98/g) and liver products from animals. The highest average levels in total product weight were for fish liver (32.6pg TEQWHO98/g) and muscle meat eel (6.7 pg TEQWHO98/g). The highest average levels were found in fish oil (10.0 pg TEQWHO98/g) expressed on 12% moisture basis. These results reflect that a large percentage of measurements exceeds different maximum levels for dioxins and dioxin-like PCBs set by legislation. However, some of these samples originated from targeted sampling during specific contamination episodes.

The EFSA report demonstrated that there is no clear trend between levels of dioxins and related compounds in food and feed over time due to changing background levels. Interestingly, fluctuations in background levels of dioxins led to increasing of dioxin concentration in some categories, while reduced concentrations were observed in other categories. Instances such as the occasional incidence of dioxin contaminations make it further challenging to discern the origin of samples, such as whether they originated from the targeted sampling protocol or random sampling protocol. The EFSA report concluded that the causal relationship between changes in background levels of dioxins and related substances in food and feed could not be established. Due to the heterogeneity of both feed and food samples, an increase in some categories and a decrease in others was observed. Other factors, such as lack of information about samples (targeted or random), make it difficult to establish a causal connection.

An Australian study (FSANZ, 2002) reported the concentrations of PCDD/Fs in selected Australian foods and concentrations in food samples collected from around the world (Table 5.2). Although such information provides a piece of critical information, yet a direct comparison between concentrations of dioxins in food across the world cannot be drawn. Several factors, such as the types of food being sampled, sampling duration, and analytical techniques used, affect the results. In particular, an adequate harmonization of the analytical procedures would improve the comparability of results.

## 3 Dioxin in fish and fish products

In fish, dioxins tend to accumulate in predators at the top of the food chain (Froescheies *et al*., 2000), and their presence is documented both in marine and freshwater fish and also in farmed and wild species. The use of fish meals and oils in aqua feeds to supply essential amino acids, fatty acids, and dietary energy is the primary input to introduce dioxins in farmed fish, while the contamination of wild fish is due to the pollution of the aquatic ecosystem.

The highest dioxin levels contamination was found in several marine fish and, in particular, in salmon (8.0 pg TEQWHO1998/g ww) and herring (7.6 pg TEQWHO98/g ww), as

*Table 5.2* Comparison of mean PCDD/F concentrations in foods across different monitoring programs.

| | *Australia (FSANZ 2004)* | *New Zealand[1,2] (MFE 1998)* | *UK (FSA 2003)* | *Netherlands[3] (Freijer* et al., *2001)* | *Europe[1] (Codex 2003)* | *North America[1] (Codex 2003)* |
|---|---|---|---|---|---|---|
| Beef | 0.0006–0.24 | 0–011 | 0.41–0.42 | 0.82 | 0.6–1 | 0.5–4.1 |
| Pork | 0.05–0.22[4] | 0–0.20[5] | – | 0.24 | 0.2–1.4 | 0.6–23 |
| Lamb | 0.004–0.25 | 0–0.07 | – | – | – | – |
| Poultry | 0.02–0.53 | 0.037–0.29 | 0.13–0.18 | 1.06 | 0.6–0.9 | 0.03–3.9 |
| Fish | 1.56–3.04 | 0.33–0.41 | 1.06–1.06 | 0.181[6,7] | 0.01–8.9[7] | 0.033–0.53[7] |
| Eggs | 0.013–0.42 | 0.017–0.12 | 0.24–0.24 | 1.52 | 0.5–2.7 | 0.044–0.3[7] |
| Milk | 0.04–0.23 | 0.019–0.16 | 0.46–0.47 | 0.57 | 0.3–2.5 | 0.3–0.9 |

1. *Results reported in I-TEQs that are 10–20% lower than WHO-TEQs.*
2. *Results reported in the range of lower to middle bound.*
3. *Results reported as lower bound only.*
4. *Assumes bacon is representative of all pork products.*
5. *Pork meat.*
6. *Carcass meat.*
7. *Lean fish.*

*Modified from FSANZ, 2002.*

reported by the EFSA study (EFSA, 2010). In particular, species coming from the Baltic Sea area reported mean values decidedly higher than those from countries not in the Baltic Sea area (EFSA,2010) (ref). Recently, it was found that the dioxin levels in the blood of Finnish fishermen consuming much fatty Baltic fish could reach the levels of exposures seen in Seveso, Italy, in 1976 (Kiviranta *et al*., 2002).

The congener-specific accumulation of dioxins in biota is a complex issue and is not entirely understood. While biomagnification exerts a definite influence on the total dioxin concentrations in biota, life behaviors seem to instead exert an influence on the differential congener-specific accumulation of dioxins (Castro-Jiménez *et al*., 2013). The PCDD/F patterns in the sea and freshwater fish tissues change with the fish species. The proportion of dioxin and furan congeners depends on their physical-chemical properties, diet, food chain biomagnification, and the lipid content of the single fish species (Lirong *et al*., 2014). For example, the 2,3,4,7,8-PeCDF is slowly metabolized in fish compared with other congeners and shows a strong age-related accumulation (Zacs *et al*., 2013).

In general, pelagic species reflect the congener pattern from the surface water dissolved phase and phytoplankton with a higher abundance of the less hydrophobic and more volatile compounds, whereas the dioxin pattern in benthic and nekton benthic species is more similar to the pattern in the deep-water dissolved phase and the sediment. Fish liver, a source of fish oil which is widely used as an accessory food substance and as a component of a wide range of pharmaceutical products, is another essential product known to have high PCDD/F levels (EFSA, 2010).

## 4 Dioxin in livestock and animal products

Dioxin levels are reported to be higher in the liver of sheep, pigs, cows, and poultry relative to muscle tissue and other organs. Once absorbed through the intestinal tract, dioxins are

initially stored in the liver before being redistributed into more lipid-rich tissues. For grazing ruminants, especially for sheep, soil intake, occurring through particles deposited on vegetables or directly by feeding on pasture herbage close to the ground surface, might be expected to contribute to sheep's exposure to dioxin to a non-negligible extent (EFSA, 2011). Again, the dioxin levels in soil itself are highly variable and often strongly influenced by seasonal variation with median soil concentration being 8% of dry matter intake. For instance, a UK study presented evidence that flooding is involved in transferring dioxins into livestock (Lake *et al*., 2014). This study showed that soil, grass, and consequently, beef meat from flood-prone farms reported dioxin levels higher (about 20%) than samples collected in control farms. Consequently, the majority of flood-prone farms (7/10) had higher median dioxin levels in beef than in the corresponding control farm.

In the past, problems with commercial feed often caused an excess of the maximum residual levels of dioxin in food of animal origin (Tlustos *et al*., 2013; Malisch *et al*., 2014), while in recent years, sheep (in particular in the liver) (EFSA, 2011) and beef (Lake *et al*., 2014) from free-range production exceeded the existing maximum limits. Recently EFSA (2011) evaluated the dioxins in 332 sheep livers and 175 sheep meat samples submitted by eight European countries. For sheep liver, the mean upper bound concentrations for dioxins (expressed as WHO-TEQ1998) were 14.9 pg WHO-TEQ/g fat (range: 0.27–116). The corresponding levels in sheep meat were considerably lower: 0.70 pg WHO-TEQ/g fat (range: 0.08–5.1). The selective accumulation in the liver is due to cytoplasmic and microsomal binding within liver tissue, such as the aryl hydrocarbon receptor (AHR) or cytochrome P450 (CYP) metabolizing enzymes. This phenomenon is common in sheep, pigs, cows, and poultry (Rose *et al*., 2010).

Studies concerning the accumulation of PCDD/Fs and PCBs in different species (Huwe *et al*., 2009), showed that poultry accumulates higher concentrations than beef or pork, caused more by individual dietary factors than by species differences in biotransformation.

Another EFSA study showed that milk at farms had higher levels of dioxins than milk from bulk (EFSA, 2012). In the lactating cows and sheep, these compounds, accumulated in the animal's fatty tissues (Schulz *et al*., 2005; Rychen *et al*., 2008), can be excreted via milk.

An Italian study describes the local distribution of pollution through the detection of high concentrations of PCDD/Fs and dl-PCBs in bovine milk. The milk collected from 27 herds was sampled throughout four years (2004–2007) in a Piedmont valley (Northwestern Italy), where environmental pollution by these substances was detected in late 2004 (Desiato *et al*., 2012). Considering as their presence in animal products could be traced back to the ingestion of contaminated fodder, the animal feed gives a significant contribution to the contamination of food (Desiato *et al*., 2014). Also, eggs contribute about 4% to the daily dioxin intake of humans, they consist of almost 10% fat, and dioxins are likely to accumulate in the fat of the yolk (Vries *et al*., 2006). In a 2012 EFSA study among the UE, farms have shown that eggs coming from battery rearing contained significantly fewer dioxins than those coming from free-range, organic, and growing outdoor production (EFSA, 2012).

Probably the critical cause of high dioxin levels in eggs is consumption by hens of contaminated worms, insect, grass, herbs, and soil. In the Netherlands, a study has shown that 25% of the 34 organic poultry farms investigated in the country produced eggs with a dioxin content that exceeded the EU standard (Vries *et al*., 2006). The distribution over tissue in laying hens is congener dependent, with 5% to 30% of the intake excreted in the eggs, 7% to 54% in animal fat, and 1% in the liver (Stephens *et al*., 1995).

## 5 Dioxins and cooking methods

Information on the levels of PCDD/Fs in food concerns primarily uncooked food; in fact, in the food survey programs, food analyses are generally performed on uncooked/raw products. However, it is well known that the physicochemical and nutritional qualities of several foods are widely modified by cooking processes, and that these modifications are also dependent upon the specific food (Choe and Min, 2007; Ferracane *et al*., 2008; Miglio *et al*., 2008). As dioxins are chemically stable, no changes in dioxin content with processing would be expected. However, possible changes in PCDD/F congener content after cooking have been reported. Hori *et al*. (2005) stated a decrease of dioxins levels in the traditional cooking process with heating applied to the animal products, while Perelló *et al*. (2010) reported that cooking processes that remove fat from the food of animal origin should tend to reduce the levels of organic pollutants in cooked food. Despite this, information concerning the effects of cooking on the increase or decrease of chemical contaminants in food is minimal (Perellò *et al*., 2010). Some studies reported that smoking meat or fish samples can increase the dioxin and furan content depending on the smoking conditions (Mayer, 1998; Mayer and Jahr, 1998). In meats, cooking seemed to reduce PCDD/F concentrations in veal steak and pork.

In contrast, reductions after cooking were not found by Rose *et al*. (2001) and Perelló *et al*. (2010). It is essential to highlight that these changes in PCDD/F concentrations appear sometime to be due to the weight changes associated with loss of water or fat or to the different methods of cooking used. Frying in animal fat might increase dioxin toxic-equivalents because of dioxins present in the fat used for frying. Keeping in mind that dioxins are associated with the fat portion of foods, cooking methods releasing fat from the product tend to reduce the total concentrations in the cooked food. It is therefore unlikely that PCDDs/PCDFs are formed or lost during usual cooking processes.

## 6 Conclusion

Dioxins are ubiquitous and persistent in the environment, and foods of animal origin are the primary source of human exposure. Human exposure to dioxins is primarily from ingestion of food high in lipid content, such as fish, meat, and dairy products. Contamination of food of animal origin is related mainly through ingestion of contaminated vegetation and soil, while fish contamination is due to the contamination of the aquatic environment. In 2002, the European Commission introduced regular monitoring of feed and food by the Member States and, in 2008, EFSA concluded that no clear trend could be established regarding changes in background levels of dioxins and dioxin-related substances in food and feed over time. It hoped for an adequate harmonization of the analytical procedures to improve the comparability of results. It is possible to decrease human exposure to dioxins using several strategies: food selection, reducing the intake of animal fat by selecting lean cuts of meat or removing skin, and by selecting low-fat dairy products.

Furthermore, the selection of cooking practices can also modify the levels of dioxins in food. The impact of cooking methods on the dioxin levels depends on the cooking process, the specific food item, and the type of compounds present. The cooking processes do not significantly modify the concentrations of dioxins, but losses of fat in preparation and cooking decrease the total amount of the chemical compounds in the finished product.

About the new strategy of dioxin control, international organizations are working to reduce the release of dioxin in the environment through control of emission sources, for example, and reducing the presence of these chemicals in entire food chains since food consumption is the most important route for human exposure. In both industrial and non-industrial countries, efforts are underway to reduce the levels of dioxins in feed and food.

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# Toxicity of dioxin

## Implication for human health & the environment

Chapter 6

# Dioxin – exposure routes, pathways, and human health implications

*Pallee Shree, Mohit Kumar, Indrakant Kumar Singh and Dileep Kumar Singh*

## 1 Introduction

Technological progress is a boon to society. It has provided significant benefits to humanity, but all have come at the cost of increasing health hazards, which comes from unwanted by-products from industries. Environmental pollution has several health impacts on humans, and it is a dreadful evolutionary challenge for all of us. In order to combat the challenge, it is a prerequisite to understand how we get exposed to pollutants and how these pollutants work inside the body at the cellular and molecular levels, as well as their clinical outcomes. Perhaps the most noteworthy example of toxic chemical agents is dioxin, which evokes a wide range of undesirable toxic effects on humans. In humans, exposure to dioxins has pleiotropic responses that have been associated with a variety of adverse effects (Pelclová *et al*., 2006; Schecter, 2013).

The term "dioxin" usually refers to a number of toxins, which share a similar chemical structure and their mechanisms of toxic actions are also common (Mandal, 2005; Bock, 2017). The complex mixture of dioxins includes 75 dioxin congeners and 135 furan congeners. Among these congeners, the most toxic contaminant of the environment in animal studies is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Poland and Kende, 1976; Denison and Nagy, 2003). Experimental evidence demonstrates that the aryl hydrocarbon receptor (AHR), which is expressed ubiquitously in several human tissues, is a ligand-activated transcription factor, responsible for toxic TCDD actions (Harper *et al*., 1991; Van den Berg *et al*., 1998; Mandal, 2005). The intoxication process is elaborate, and the aryl hydrocarbon receptor (AHR) is vital for its functioning (Bock, 2017; Kulkarni *et al*., 2008).

Accidental or intended formation of these chemicals inevitably finds its way in humans, via different pathways. Universally renowned among these routes of dioxin intoxication to humans is the ingestion of contaminated foods, like milk and meat (Malisch and Kotz, 2014; Kulkarni *et al*., 2008; Sapkota *et al*., 2007), pollution (Kampa and Castanas, 2008), incineration of wastes, metal industries (Danjou *et al*., 2015), paper and pulp industries, and use of chemical herbicides (Kogevinas, 2001). A successful initiation of the intoxication process instigates a variety of chronic or fatal outcomes, such as cancer (Xu *et al*., 2016; Villeneuve *et al*., 2010; Kogevinas, 2001), impaired development of the nervous system and loss of brain functions (Xu *et al*., 2017), dermatological issues (Kogevinas, 2001; Sweeney and Mocarelli, 2000), and liver issues (Harvey *et al*., 2016).

## 2 Human exposure to dioxin

Human exposure to dioxin poisons occurs in a variety of ways that all fall under erroneous or deliberate fabrication of the poison (Mandal, 2005; Watanabe, 1999). The deliberate manufacture of dioxin toxins and subsequent exposure caused huge damage to humans, which led to bans of these polychlorinated biphenyls (Malisch and Kotz, 2014). Unintentional exposure to dioxin poisons, on the other hand, comprises accidental circumstances, occupational, or it can be via environmental exposure. Such unfortunate situations include incomplete combustion of chlorinated substances originating from metal and cement industries, incineration of waste, and some domestic activities (Danjou *et al*., 2015; Kulkarni *et al*., 2008). Diet is also regarded as one of the primary sources of dioxin given that its components are enormously lipophilic and constitute persistent pollutants that contaminate food (Danjou *et al*., 2015). Regarded as by-products of accidents, dioxins are produced when substances containing carbon, oxygen, hydrogen, and chlorine are heated.

### 2.1 *Environmental exposure to dioxin*

Waste incineration is the primary form of environmental dioxin production, with the combustion process emitting dioxin toxins that evade capture by the system in place (Shibamoto *et al*., 2007; Kulkarni *et al*., 2008). Well-known ways of waste incineration that are highly responsible for the release of dioxin to the environment are: i) incineration of municipal solid wastes, ii) incineration of hospital wastes, which consist of burning both human and non-human wastes, iii) chemical or hazardous waste incineration, and iv) incineration of solid wastes from sewage treatment (Singh and Prakash, 2007; Kulkarni *et al*., 2008). Combustion processes that release dioxin into the environment are diesel vehicles, coal burning, wood burning, cremation, cigarette smoking, exhaust from automobiles, electric steel-making furnaces, the use of waste as fuel for cement kilns, municipal solid waste incinerators, etc. (Liem and van Zorge, 1995). Kulkarni *et al*., 2008; Nzihou *et al*., 2012; Dwyer *et al*., 2015). Many studies conducted in contaminated areas, like near power plants or incineration sites, concluded that people who lived in those areas have the highest level of dioxin in their bodies. Chen *et al*. (2006) observed that there is an increase in the body burden of dioxins in pregnant women residing near a power plant as compared to the general Taiwanese population in an area of Tainan city. Danjou *et al*. (2015) reported that in France, women residing in proximity to a municipal solid-waste incinerator were found to have a high level of dioxin in their breasts. In Michigan, complaints were raised about the potential effects of dioxin discharge on a population group exposed to it due to the production of dioxin by Dow Chemical Company in the Midland (Regenstein, 1983).

### 2.2 *Accidental exposure to dioxin*

Cases of accidental exposure to dioxin and its effects reported in the past, wherein individuals got exposed to dioxin due to accidents that happened at a workplace or near residential communities, especially those located near plants or manufacturing units, or even due to natural accidents, like forest fires and volcanic eruptions, are also thought to emit dioxin into the environment (Kim *et al*., 2003; Srogi, 2008). Dioxins are accumulated in reservoirs and present in microbial release (Kumar *et al*., 2013. Kulkarni *et al*., 2008). One of the notorious descriptions of human accidental exposure to dioxin-contaminated chemicals happened following an explosion in a trichlorophenol reactor in Nitro, West Virginia, (USA) in the year

1949 (Sweeney and Mocarelli, 2000). Another well-known case among these studies is the accidental exposure of an entire community in Italy to dioxin following the 1976 Seveso accident, which exposed several thousand people to substantial quantities of TCDD (Bertazzi *et al*., 2001; Danjou *et al*., 2015). Subsequent follow-ups to the Seveso accident were performed and they revealed the persistent presence of dioxin expected, given its long half-life in humans (Pesatori *et al*., 2009; Baccarelli *et al*., 2002; Bertazzi *et al*., 2001). Another significant accident was reported in Missouri (USA) in the 1970s, where to control dust, oil contaminated with dioxin was sprayed on streets. The contamination of the oil was discovered by the EPA (Environmental Protection Agency). After that, the U.S. government ordered the town cleaned, and it was evacuated (EPA, 2010).

Another form of accidental discharge of dioxin into the environment is through the release of brominated dioxin. Little is known about its effects and mechanisms of toxicity, but it reportedly is released upon burning plastic materials that contain bromine flame retardant (Government of Japan, 2012). The presence of bromine was first validated in the Netherlands after failure testing for chlorinated dioxin. Although the pathways could not be accounted for, the certainty was there, and precautions were taken (Malisch and Kotz, 2014).

### 2.3 *Industrial exposure*

The majority of dioxin impacts on humans are reported among highly exposed groups, which include occupational populations who deal with pesticide application, chemical production, and persons who handle or get exposed to materials that contain dioxin as a contaminant or contaminated pesticides. The chlorination of naturally occurring compounds, such as pulp used for manufacturing paper, is also known as an industrial form that produces dioxin (Bock, 2017; Kulkarni *et al*., 2008). Other potential sources of dioxin is metallurgical industries which deal thermal metallurgical process such as the production steel at very high temperatures, sintering of iron ore, metal smelting operations etc. (Kulkarni *et al*., 2008; Buekens *et al*., 2001). The use, in the past, of some chemical substances to treat soils and waters has also been mooted as a possible way to have let dioxin into the environment (Kulkarni *et al*., 2008). Industrial exposures also occur in workers involved in manufacturing and processing chemicals, phenoxy herbicides, and chlorophenols applicators (Boers *et al*., 2010; Manz *et al*., 1991). Occupational exposure incidence of 2,3,7,8-TCDD has been reported among those factory workers involved in handling 2,4,5-T and 2,4-D. The first prevalence of industrial exposure was reported in 1949 among workers of a 2,4,5-T producing factory in West Virginia (Zack and Gaffey,1983; Sweeney and Mocarelli, 2000). Villeneuve *et al*. (2010) reported substantial levels of dioxin in male workers of various professions, which included motor vehicle mechanics, paper makers and painters, furniture manufacture, health, social workers, forestry, and logging workers. In addition, the test for the presence of dioxin as a male breast cancer causative agent also implicated dioxin as a potentially cancer-causing chemical (Xu *et al*., 2016). Occupational exposure to dioxin or TCDD most likely occurs through inhalation of TCDD-contaminated particles/dust and via dermal contact with products or solutions containing TCDDs.

### 2.4 *Residential exposure*

Not only are people contaminated via direct contact with dioxin-related chemicals or dioxin-contaminated materials, through accidental or industrial exposure, they also get exposed to the chemical through residency. People living in areas where industrial

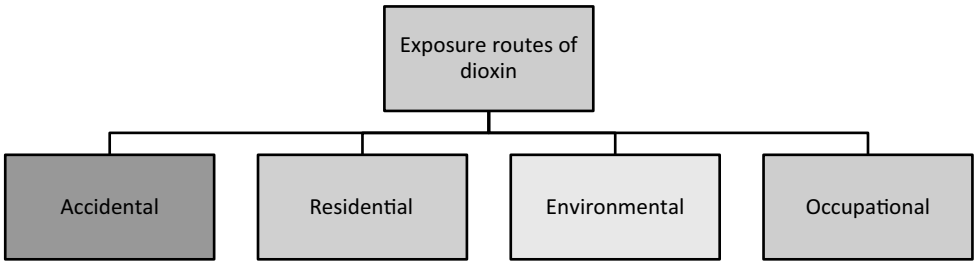


*Figure 6.1* Exposure routes of dioxin into humans.

works, mechanical works, incineration, and combustion occur are also liable to accumulate dioxin in their systems (Pronk *et al*., 2013). Dioxin exposure studies conducted by the University of Michigan revealed that discharge of dioxin from the Dow Chemical Company in Midland, Michigan, increased body burdens of dioxin in residents when compared with a reference population. Studies eventually carried out on household dust and soil concluded that nearby areas had higher concentrations of dioxin, which resulted in higher dioxin concentrations in blood samples collected from residents from these places and who had eaten of the fish from the aerial waters. (Hedgeman *et al*., 2009; Franzblau *et al*., 2010). Nestrick *et al*. (1986) reported that residential wood combustion was found to be a noticeable source of TCDD emissions. Residential exposure mainly occurs by means of dietary fat, predominantly milk, meat, fish, and other animal products (Kogevinas, 2001).

## 3 Pathways of human exposure to dioxin

Dioxins are persistent organic pollutants (POPs), which are considered to be persistent in the environment as they have very long half-lives in different media like air, soils, water, and biota (Jones and De Voogt, 1999). They are released via various natural as well as anthropogenic sources in different media as mentioned above. From any of these three primary media, it gets re-deposited onto biota. The physical state and chemical properties of dioxin-like lipophilic character dictate its deposition pathway. The atmosphere is the most abundant reservoir of dioxin releases; therefore, it is imperative to know how dioxins get transferred from the atmosphere into flora and fauna. In spite of atmospheric concentration, dioxin is also present in soil and water. Now, the question arises, how does it bioaccumulate in humans? The accumulation of dioxin in humans occurs directly from a reservoir or via secondary sources that are food (animals and plants). Bioaccumulation of dioxin occurs in the plants via soil, water, and atmosphere. The maximum contribution occurs through the atmosphere. The dioxin which is bioaccumulated in plants leads to the entry of dioxin into the food chain and gets accumulated in body fats of humans and animals. When it enters into the human body, it leads to several diseases, and its degradation in human bodies depends on several factors like age, sex, fat in the body, the half-life of dioxin in humans, and smoking habits. The complete pathway of dioxin from the reservoir to the human body is depicted in Figure 6.2.

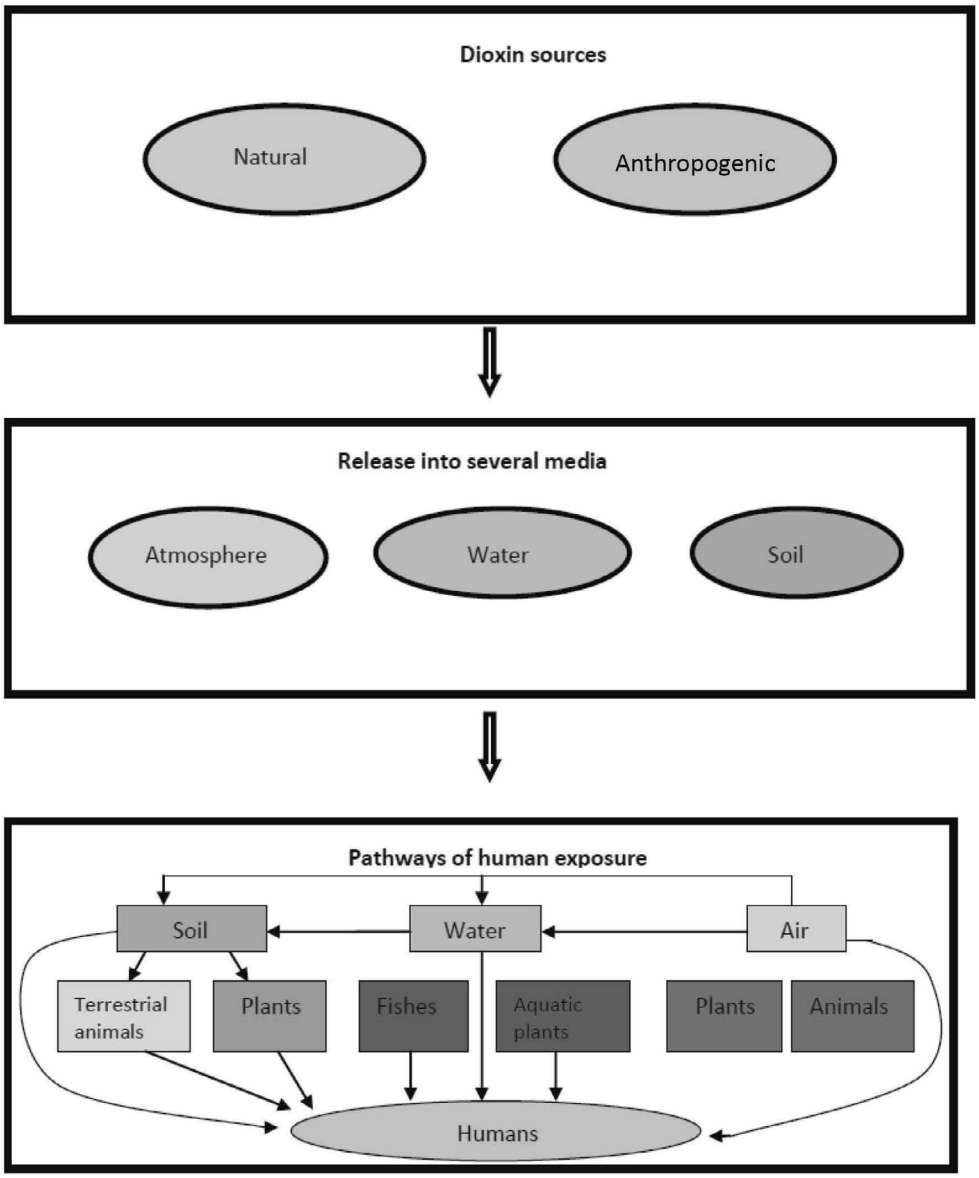


*Figure 6.2* Dioxin pathway of human exposure from various sources.

## 4 Through food

Diet is one of the predominant ways in which dioxin finds itself into the human system. Foods responsible for dioxin exposure are fatty foods because dioxin is soluble in lipids (Goncharov *et al*., 2008). Most culpable of foods are seafood, dairy products, eggs, and meat (Danjou *et al*., 2015; Malisch and Kotz, 2014; Kulkarni *et al*., 2008; Kogevinas,

2001; Kulkarni *et al*., 2008). Continentally, fish constitutes the primary source of dietary dioxin intake in Japan and Europe (north Germans and south Scandinavians), while meat and dairy products are predominant in some parts of Europe and the USA (Government of Japan, 2012; Tsutsumi *et al*., 2001; Kogevinas, 2001), so humans are primarily exposed to dioxin via contaminated fish and meat. When elevated levels of dioxin were detected in cow's milk in Germany, three studies in two continents had to be carried out to eventually uncover that the root of the issue originated from Brazilian pulp pellets fed to the cows. Ball clay is another source of dioxin implicated in contaminating chicken (Malisch and Kotz, 2014). Food from these animals was reported to have caused human contamination. High levels of dioxin have also been traced in human breast milk, which is contracted from eating dioxin-contaminated food (Abraham, 2017; van den Berg *et al*., 2017). Exposure to contaminated feeds for chicken and consumption of the chicken, as well as other animals, demonstrates how quickly and ignorantly humans can be exposed to dioxin via food. This chemical gets stored in the human adipose tissues (Danjou *et al*., 2015). Once in adipose tissues, the rate of dioxin disposition is annoyingly slow, requiring a minimum of seven years to reduce it to half its original value (Kogevinas, 2010; Government of Japan, 2012). The insolubility property of dioxin is purportedly accountable for its non-biodegradable state (Kulkarni *et al*., 2008). It has subsequently been revealed that bioaccumulation from feed to food of animal origin changes the dioxin pattern considerably (Malisch and Kotz, 2014). However, only the consumption of very large amounts would be required for any alarms to be raised, as only tiny traces of this chemical are freely available in the atmosphere (Government of Japan, 2012). The tolerable daily intake of dioxin is 4 pg for each kg of body weight per day (Saurat *et al*., 2012), but it has been estimated that only 0.85 pg is taken in per human every day, a value far below the tolerable mark (Government of Japan, 2012). Also, the data indicate that exposure to oral routes via low levels of dioxin-contaminated food and milk represents a principal way of exposure for people (Schecter *et al*., 1994).

## 5 Through inhalation

Another form of exposure to dioxin has been through the handling of materials of chemical production, through pesticide users, and people who have handled and been exposed to other dioxin-contaminated materials (Sweeney and Mocarelli, 2000). Dioxin escapes into the atmosphere by different natural or anthropogenic activities which cause adverse impacts on health. Most pertinent to the inhalation of dioxin is breathing contaminated air. Combustion of fossil fuels is mainly responsible for the progressive increase in concentrations of dioxin in the atmospheric. Several studies conducted revealed that the levels of dioxin inhaled depended on the level of atmospheric contamination. which has the potential to impact on health proportionally (Paustenbach *et al*., 1991). In addition, testing for dioxin levels inhaled following household heating with coal in the winter period revealed that inhaled dioxin levels in humans increased to about 6.5-fold higher than in the summer and could be a significant means of dioxin intoxication (Dziubanek *et al*., 2016). Dioxin has also been found in women's breast milk that was highly exposed to dioxin and dioxin-related substances (Abraham, 2017; van den Berg *et al*., 2017). Reportedly, this exposure could be neurologically damning to newborns with the vernix caseosa of a fetus tipped as the primary site of excretion, due to its high content in dioxin (Tran *et al*., 2016; Morokuma, 2017).

## 6 Through dermal path

Most contact exposures to dioxin are dermal, especially in workers who directly manipulate dioxin-related chemicals or dioxin-contaminated materials. The dermal manifestation of dioxin is mainly through skin lesions, otherwise known as chloracne (Saurat *et al*., 2012; Mitrou *et al*., 2001; Sweeney and Mocarelli, 2000). Chloracne is also said to be the most visible and consistent response to dioxin exposure (Suskind, 1985; Saurat *et al*., 2012). Their handlings have led to concerns (Kogevinas, 2001). In the case of an individual poisoned with 5 million-fold more than the accepted daily intake, five years of molecular medicine observation approach was adopted, and results revealed that skin lesions, later found to be hamartomas, covered up to 40% of the patient's surface body (Saurat *et al*., 2012). Among other reported cases are a few with and without additional effects in workers at companies producing dioxin-contaminated products, workers exposed to pure dioxin, and children who live in Seveso (Sweeney and Mocarelli, 2000). Other reported dermal conditions from past investigations include conjunctivitis, eyelid cysts, inflammation of eyelids, irritated and red eyes, actinic elastosis hyperpigmentation, and Peyronie's disease (Sweeney and Mocarelli, 2000).

## 7 Others

Amid reports of dioxin infection through tampons and diapers in San Francisco, tests were carried out, and results eventually proved that contamination via the use of tampons and diapers was insignificant (DeVito and Schecter, 2002).

## 8 Mechanism of metabolic pathway

The instigator of the dioxin route to the human system is the aryl hydrocarbon receptor (AHR), which is a multifunctional transcription factor of the PAS (Per-Arnt-Sim) superfamily. It is the only ligand member of the family to undergo activation (Bock, 2017). AHR is known to be a key mediator in the transcriptional regulation of dioxin-cause altered genes, helping facilitate the mechanism of action of dioxin in humans (Xu *et al*., 2017; Marinković *et al*., 2010; White and Birnbaum, 2009; Kulkarni *et al*., 2008; Mandal, 2005). It is believed to be highly conserved in different species, with the route of human exposure having precious little influence on its effects (Kogevinas, 2001). The liaison between AHR and dioxin takes place in the following way: (i) AHR is an intracellular receptor and transcription factor which binds to a variety of ligands including dioxin, and in the cytosol this receptor is attached to small protein (p23), two heat shock proteins (HSP90), and an immunophilin-like protein (XAP2) (Beischlag *et al*., 2008; Mimura and Fujii-Kuriyama, 2003); (ii) Binding of ligand (dioxin) to AHR leads to its dissociation from the complex of proteins and AHR forms the dioxin-receptor complex; (iii) The dioxin-bound receptor then undergoes nuclear translocation and couples with AHR nuclear translocator (Arnt); (iv) The heterodimer that is AHR-ARNT acts as a transcription factor, which modulates expression of the gene by binding to the dioxin responsive elements present that lie at 5′ promoter regions of Aryl hydrocarbon-receptor in a bit to manipulate gene activates of xenobiotic metabolizing enzymes, like CYP1A1 and CYP1B1 or cytochrome P4501A1 and P4501B1 together with it, it also manipulates other genes

expression (Xu *et al*., 2017; Marinović *et al*., 2010; White and Birnbaum, 2009; Whitlock, 1999); (v) Protracted CYP1A1 gene expression may increase the probability of deleterious DNA lesions; this augmentation might occur because of an increase in the production of genotoxic metabolites and (ROS) reactive oxygen species. Similarly, the CYP1B1 gene might be responsible for cancer as it has been reported that it might be involved in the mechanism of carcinogenesis via concerned in the metabolism of 17-estradiol as well as bio-activation of several arylamines and polycyclic aromatic hydrocarbons. Therefore, it can be concluded that the exact mechanisms of carcinogenicity and other health impact are currently unknown, but probably most of the toxic effects caused by dioxin are mediated via AHR.

This dangerous chemical reportedly is associated with the gene expression of cytochrome gene P450A, P450B isoforms or CYP1A1, and CYP1A2, which leads to defects in the reproductive and developmental systems, immunotoxicity, modulation of growth factors and their receptors, and the disruption of normal hormone signaling pathways (Emond *et al*., 2016; Marinković *et al*., 2010; Watanabe, 1999; Mandal, 2005). It has recently been revealed that genes related to neurotransmission, neurodevelopment, and cytotoxicity are important for the mechanism of dioxin effects as well (Xu *et al*., 2017). Although a human carcinogen, dioxin would be required in huge amounts to cause severe medical conditions. However, accidental situations in the past and other investigations have proven that even the little traces consumed could be detrimental to human health, and thus caution must be taken in dealing with the chemical.

## 9 Impact of dioxin on human health

Incidents of dioxin in humans are numerous, from dermatological to cancerous. Cancerous situations have been noted to have increased with exposure to dioxin. Kogevinas (2001) reported as high as 50% increased rates of cancer in studies carried out among workers exposed to dioxin. Danjou *et al*. (2015) also laid bare the effects of this carcinogenic chemical on cancer incidence rates in their report of past evaluations among children and men. The identification of high breast cancer incidence in male workers of various professions after tests for the presence of dioxin as a male breast cancer causative agent also implicated dioxin as a potentially cancer-causing chemical (Xu *et al*., 2016; Villeneuve *et al*., 2010), and has since been classified a human carcinogen.

Non-cancerous effects are also plenty to have gripped workers or individuals exposed to dioxin. Chief among these incidents are dermatological effects (Kogevinas, 2001; Sweeney and Mocarelli, 2000). Chloracne is a dermal effect that has arisen from human exposure to dioxin-contaminated substances (Mitrou *et al*., 2001). Cases of this effect have been reported in many investigations. Among the reported cases, there are the workers of TCP production facilities, workers at companies producing dioxin-contaminated products, workers exposed to pure dioxin, and the child who lives in Seveso (Sweeney and Mocarelli, 2000). Dioxin has also been isolated from breastfeeding milk of women highly exposed to dioxin and dioxin-related substances (Abraham, 2017; van den Berg *et al*., 2017) and reportedly could be neurologically damning to newborns (Tran *et al*., 2016), with the vernix caseosa of a fetus tipped as the main site of excretion, due to its high content in dioxin (Morokuma, 2017).

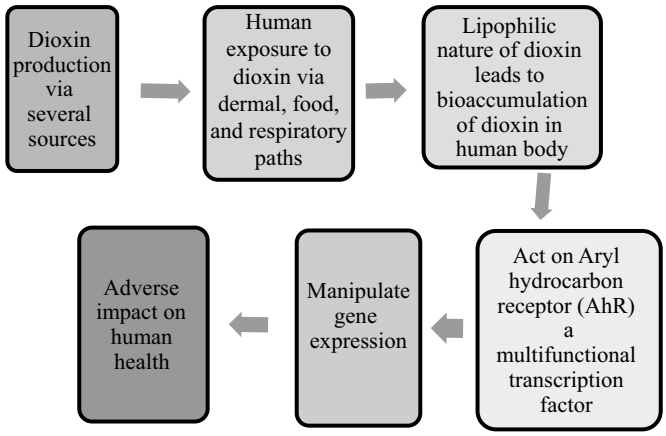


*Figure 6.3* Sources of dioxin exposure, its mode of action and human health.

Other mentioned but significantly unimportant events are the presence of dioxin in human reproductive systems, allegedly responsible for low testosterone and high gonadotropin concentrations exposed to dioxin, diabetes (with high glucose rates but low insignificant incidence rates), and thyroid function (Kogevinas, 2001). Mitrou *et al*. (2001) also reported many health problems associated with dioxin. These include soft tissues, sarcomas, lymphomas, immune system and neurological effects, liver issues, elevated blood lipids, and stomach cancer.

A summary of exposure pathways is discussed above through which it enters into the body. Most of the health impacts of dioxin mediated via AHR receptor but there can be several other pathways of dioxin action (Figure 6.3). Although a human carcinogen, vast amounts of dioxin would be required to cause severe medical conditions. However, accidental situations in the past and many other investigations have proven that even the little traces consumed could be detrimental to human health, and thus caution must be taken in dealing with the chemical.

To study the impact of dioxins on human health, several epidemiological studies of exposures have been carried out, like the case of industrial workers and industrial accident herbicide application. But studying the human health impact is complicated as knowledge of exposure data and simultaneous exposure to other compounds containing traces of dioxin, such as smoking, is incomplete. Another problem in studying human health impact is finding the same individuals who participated in an earlier study so that long-term effects can be looked at. Another problem in analyzing the data is there is limited knowledge about the amount of exposure and how much the person is exposed to which other chemicals. In spite of all these obstacles, several case studies on different population size to examine the impact of dioxin on human health and significant health impact are mentioned below (Figure 6.4).

The exposure of dioxin can be short-term or long-term, which creates several different types of impact (Figure 6.5). The acute/short-term exposure of high level of dioxins to humans may lead to dark patches or lesions on the skin and chloracne, which is considered as the hallmark of dioxin exposure and also occurs in chronic exposure (Otles and Yildiz,

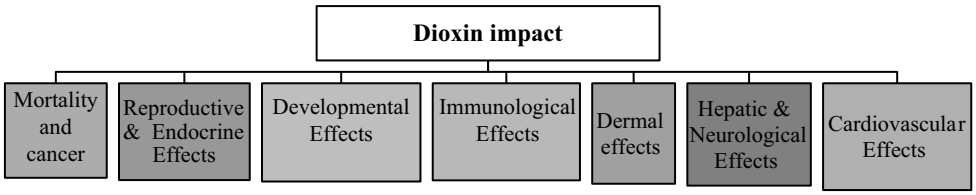


*Figure 6.4* List of impacts of dioxin on humans.

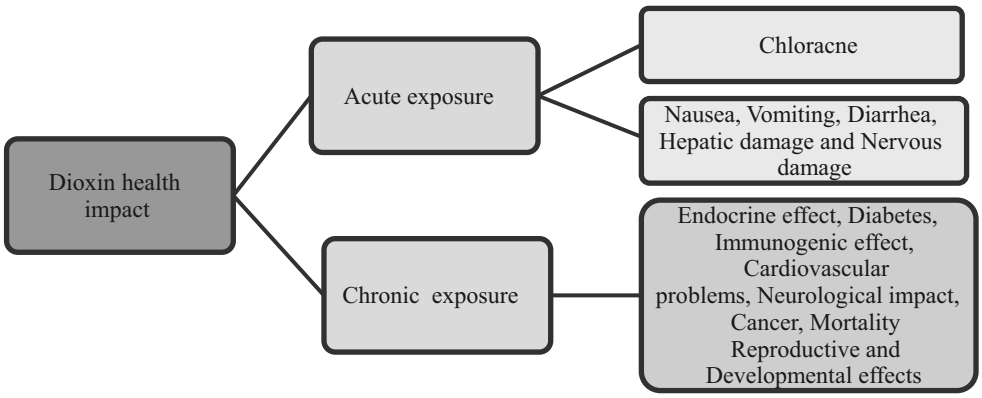


*Figure 6.5* Chronic and acute dioxin exposure and their impacts.
(*Sources*: PHE, 2008; Otles and Yildiz, 2003; Suskind, 1985)

2003). It has also been reported that acute exposure to dioxins may result in general toxicity nausea, diarrhea, vomiting, liver, and neurological malfunction (Suskind, 1985; PHE, 2008). Long-term/chronic exposure may lead to chloracne, developmental disorders, neurotoxicity, hepatotoxicity, cancer, mortality, and it has also adverse impacts on the function of reproductive, immune, endocrine, and reproductive system functions (Chahoud *et al*., 1989; Fan and Rozman, 1995; Sweeney and Mocarelli, 2000; Mitrou *et al*., 2001; Pavuk *et al*., 2003; Hogaboam *et al*., 2007; Halldorsson *et al*., 2009; Leijs *et al*., 2008), endometriosis (Eskenazi *et al*., 2000; Heilier *et al*., 2007), and increased risk for diabetes (Longnecker and Michalek, 2000). Chronic exposure of animals and humans to dioxins has resulted in several types of cancer. Therefore, dioxin was considered as a potential human cancer-causing substance by the International Agency for Research on Cancer (IARC) in 1997.

## 10 Effects on the immune system

Limited studies on humans suggest some severe outcomes of dioxin exposures on the human immune system, which can affect resistance to infectious disease caused by suppressed cell-mediated and humoral immune responses (Birnbaum and Tuomisto, 2000; Lawrence and Kerkvliet, 2006). The impact on the immune system leads to a severe disturbance on

human health and, as of now in the industrial era, it's a serious concern as the concentration of dioxin is increasing progressively. Dioxin decreases immunoglobulin (Ig) production and in turn reduces resistance to viruses, bacteria, and parasitic infections (Jeong, 2017; Fiorito *et al*., 2017; Birnbaum and Tuomisto, 2000). TCDD has an impact on immunity, which leads to declining in IgG levels, total γ-globulin, and serum IgM (Neubert *et al*., 2000; Sulentic *et al*., 1998; Løvik *et al*., 1996; Jansing and Korff, 1994). A case study was done on a random population residing near a highly exposed zone nearly after 20 years after the Seveso city (Italy) accident and it was reported that IgG levels in the exposed population decreased as compared with unexposed people from an uncontaminated area (Baccarelli *et al*., 2002). In another study on a dioxin-exposed population of Seveso, soon after the accident, no change in Ig concentrations was reported (Pocchiari *et al*., 1979). Other reports showed no alterations in immunoglobulin levels (Jennings *et al*., 1988; Jung *et al*., 1998; Michalek *et al*., 1999; Leijs *et al*., 2009). A slight increase in IgG levels was found in two studies (Ott *et al*., 1994; Webb *et al*., 1989). All these reports and studies suggest the role of dioxin in immune compromisation. The Air Force Ranch Hand study showed an increased IgA level of dioxin in exposed individuals. This increase in IgA levels entirely correlates the effect of dioxin on the human immune system (National Research Council, 2006). Michalek *et al*. (1999) studied immune response among veterans of Operation Ranch who were involved in aerial spraying of herbicides from 1962–1971. They observed no consistent relation between alteration in the immune system and dioxin exposure.

Zober *et al*. (1994) reported in their cohort study that dioxin-exposed workers have an augmented level of attack by infections and parasitic diseases; however, the clinical diagnoses were non-specific or ill-defined. Previous studies predicted alteration in humoral immunity, but many researchers reported no consistent relationship among them (Neubert *et al*., 2000; Hosnijeh *et al*., 2011). Dioxin impacts the immune system; one of the most noteworthy impacts is thymic atrophy in several species as the thymus is responsible for cell-mediated immunity (Schecter, 2013; Laiosa *et al*., 2003). The P27 (Kip1) gene mediates thymocyte proliferation. Exposure of dioxin alters the Kip1 transcription, which induces p27cyclin/CDK inhibitor, which leads to thymic atrophy, and the above is confirmed in a rat model (Watanabe *et al*., 1999). Augmentation in the occurrence of infections in the upper respiratory tract was noticed in dioxin-exposed workers, 35 years after the BASF accident (Zober *et al*., 1994).

As for the complement components, one investigation showed a positive association between blood TCDD and C4 (Ott *et al*., 1994). Increases in C3 and CH50 also were reported elsewhere (Halperin *et al*., 1998; Sirchia, 1982). A similar study revealed a weak positive correlation between C4 and dioxin plasma levels. However, there was no significant association that existed after adjusting for age, sex, and various other probable confounding factors (Baccarelli *et al*., 2002). Leijs *et al*. (2009) reported that a cohort of mother and baby pairs were observed to detect any abnormality in children with relation to dioxin levels in the mother's milk. Immunological and hematological effects of dioxin were studied at birth till adolescence. No association between thrombocytes, leukocytes, thrombopoietin, and hemoglobin levels and dioxin were observed. Enhanced WBC counts were reported in the Missouri population (Hoffman *et al*., 1986). However, a follow-up report of the same population showed no dissimilarity observed between the count of RBC, WBC, and platelet among exposure, and non-exposure (Watanabe *et al*., 1991).

Studies on rat models showed dramatic changes in the suppression of the immune system due to dioxin exposure. However, in human studies no significant observation

has been noticed. Rather, the increased T4/T8 ratio according to the plasma dioxin level is observed (Neubert, 1991). Jennings *et al*. (1988) examined immunological parameters 17 years after an accident in the works of a 2,4,5-T factory located in Coalite, England. He found a significant increase in the natural killer (NK) cells population, an enhanced concentration of antinuclear antibodies and other immune complexes.

## 11 Effect on reproduction

Dioxin is a toxic persistent environmental pollutant. Exposure to it results in several health hazards. It causes developmental and reproductive damage as well. Risks observed are reproductive cancers, including the development of endometriosis. It has also impacted sperm quality, sperm count, the normal process of spermatogenesis, epididymis, and endocrine system. Several studies on animals showed that endocrine, reproductive, and developmental effects are among the most sensitive to dioxin exposure. Several cohort and case studies concluded that dioxin has an impact on sperm concentration, an irreversible decrease in estradiol concentration, and an increase in FSH. Dioxin also impacts the pathobiology of endometrosis development by modulating the endocrine and immune functions.

### 11.1 *Effect on endometriosis development*

Dioxins and dioxin-like substances are toxic to human health, and they are concerned with severe human health issues. The causative agent of this disease is estrogen imbalance as well as changes in the immune system. Several studies on environmental contaminants imply that they play a significant role in the endometriosis pathobiology. Previous studies on non-human primates and animal models depicted a correlation between exposures to dioxin with an amplified prevalence of endometriosis. Endometriosis is a gynecological infertility disorder which affects many women of reproductive age (Holoch and Lessey, 2010). De Felip *et al*. (2004), in their pilot case-control study on 18 Belgian and 22 mature Italian women of reproductive age with and without endometriosis, observed no significant differences found in dioxin-like compound body burdens and endometriosis between cases and controls. Mayani *et al*. (1997), in their studies on an Israeli population, reported most of the infertile women suffering from endometriosis had a detectable level of dioxin as compared to infertile women without endometriosis. Mayani *et al*. (1997), on the basis of study, concluded that dioxin concentration is directly correlated with endometriosis development and severity in humans. Gu *et al*., in the year, 2009, concluded in his report that there is insufficient evidence till his study date which can support the hypothesis that exposure to dioxin could be a cause which may lead to increased risk of endometriosis. A recent review by Sofo *et al*. (2015) concluded, on the basis of previous findings, that dioxin could modulate multiple levels of transcription. They found there is evidence of high dioxin levels and endometriosis. But recent epidemiological studies on humans can't provide a clear picture of the relation between them. Therefore, the connection between dioxin and endometriosis is still controversial.

### 11.2 *Effect on endocrine system*

Dioxins have adverse and toxic effects on human health, especially on the endocrine system. Dioxin exposure can lead to pregnancy disruption, as it is linked to the uterine

insensitivity of the ovarian progesterone hormone. Dioxin exposure also leads to delays in breast development in girls (Den *et al*., 2002). Due to this, humans may face infertility, sustained pregnancy, and even abortion, as is proved in a murine model. Dioxin can promote and enhance the development of endometriosis in humans (Sofo *et al*., 2015). Their exposure can epigenetically modify the genes related to the human diseases and the early exposure outcomes are seen after many years. Due to dioxin exposure, epigenetic changes (methylation) in genetic material and loss of gonadotropin hormone receptors, like progesterone receptor (PR), are also reported (Marques *et al*., 2013). These progesterone receptors are important molecules of the human endocrine system and alteration in that may lead to adverse impacts. The literature suggests early-life exposures of dioxin impact the functionality of the adult uterus. Certain residues in dioxins, like polychlorinated dibenzofurans (PCDF), dibenzo-p-dioxins (PCDD), and polychlorinated biphenyls (PCB), can accumulate in the human body and also contaminate the mother's milk. PCDD, PCDF, and PCB hence ultimately reached the newly born baby via contaminated milk (Marinković *et al*., 2010; Someya *et al*., 2010). Therefore, in newly born babies, the progenitor stem cells get exposed to these chemicals as their half-life ranges between five to seven years (Boitano *et al*., 2010)). Accumulated dioxin in babies creates endocrine imbalance, which further leads to abnormal endocrine system development in the human body and thus, it also leads to the disruption of gonadal development and gonads activity (Mocarelli *et al*., 2011; Foster *et al*., 2009). Studies have shown that hormonal imbalance occurs in the human body if it gets exposed during the early stage of development. A study done on residents exposed in 1976 accident in Seveso, Italy to investigate sperm quality and reproductive hormones in exposed males proved that dioxin could lead to reduced sperm motility, reduction in sperm count, and an increase in the follicle stimulating hormone (FSH) in humans (Mocarelli *et al*., 2007). Estradiol is an essential and important gonadal hormone for the humans. Exposure to dioxin both at an early stage or at puberty can permanently reduce the estradiol hormone (Brunnberg *et al*., 2011). Decreased sperm quality can often be seen in young men who reside in industrialized areas or are continuously exposed to the TCDD (Foster *et al*., 2011). Hence, a hormonal milieu of the human body is affected by dioxin. Dioxin exposure and its correlation with the variability of secretion and function shows the endocrine disruption ability of dioxins (Kogevinas, 2001). But interestingly, consistency in data has not been shown (Sweeney and Mocarelli, 2000). In another study of the National Institute for Occupational Safety and Health (NIOSH), there was no significant change in the data statistics of exposed and non-exposed cohorts of humans with the exposure of dioxins. Alterations in function of the thyroid gland were occasionally found. Elevated thyroxin in the free thyroxine index was observed in workers in the TCP unit area when industrialization was at its peak (Calvert *et al*., 1999). Similarly, studies reported dioxin is related with the slight increase in TSH concentrations, which proves the disruption of endocrine hormones and chemicals due to dioxin exposure, although no data were statistically significant (Wolfe *et al*., 1992). Estrogen reprogramming in the developmental process of human life, due to the continuous, disturbed, or accidental exposure of dioxins, can lead to the decreased and permanent alteration of function of the prostate gland during aging (Hu *et al*., 2012). Dioxins may affect the estrogenic activity, and there are scientifically proven reports which support the risk of increased prostate cancer (Chamie *et al*., 2008). Dioxins can act as an estrogenic chemical and can influence the development of genetic changes during the early development of the prostate gland by transforming and reprogramming the genetic materials. Stem

cells and progenitor cells are the targets of these contaminants in our environments (Boitano *et al*., 2010). As estrogen is required for gonadal developments, the possibility of the estrogenic activity on gonads for endocrine disruption and cancer initiation cannot be denied. Human studies are comparably less compared to animal model studies, but available studies are sufficient to support the endocrine disruption activity of dioxins. A novel prostasphere model was developed by using the prostate stem cells, and it was observed that these cells had estrogenic receptors (ERs) and were the direct targets of estrogen action (Denison *et al*., 2011). In addition to this, it was also revealed that estrogen initiates the carcinogenesis of prostate progenitor cells in combination with an androgenic environment. These kinds of studies always supported the hypothesis that dioxins or other EDCs have the potential to disturb the hormonal balance of human body and can be reprogrammed and reconstruct the stem/progenitor cells during the early stage of development.

## 11.3 *Effect on spermatogenesis sperm quality and sperm count*

Besides the impact on endometriosis, sperm count is also affected by dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity and is confirmed in the male rat when they were given in-utero exposure to dioxin. Due to dioxin toxicity, a decrease in the epididymal sperm count was noticed in adult rats. There are many conflicts in reports about the reduced sperm count as different laboratories have different observations about dioxin exposure and toxicity on reproductive organs. TCCD at higher concentrations can reduce the cauda sperm counts in animals; hence, the is more concern for human health. The process of spermatogenesis is similar in rats and humans; thus, dioxin pollution and effects on human health are noticeable in the current industrialized situation. Among the reproductive/developmental effects documented in the literature, spermatogenesis, and sperm counts are most affected and the latter shown to be decreased by up to 36% compared with controls (Bjerke andPeterson, 1994). AHRs are the receptors for dioxin interactions, and the reproductive effects of TCCD vary due to the severity of TCCD exposure and expressivity of different AHR alleles in mammalian testes, semnal vesical, vasa difference, prostate gland, and epididymis. Dioxins cause apoptosis of spermatocytes. Galimova *et al*. (2015) reported that the contamination of the semen of infertile persons by dioxin supports the hypothesis that there exists an association between dioxin and other environmental contaminants with reproductive health. In a previous study on population impacted by the plant explosion near Seveso, Italy, it has been observed that male reproductive systems are more sensitive to perinatal and in *utero* exposure to dioxin. They reported that 39 men exposed postnatally, *in utero*, and during breastfeeding had a significantly lower concentration of total sperm count (Mocarelli *et al*., 2011). In the past decades, young adult men from the industrialized area have been reported to have a low and decreased semen quality and sperm count, which is a yardstick of male fertility. Mocarelli *et al*. (2011) also study the impact of a small dose of dioxin on sperm quality. A previous study was carried by Mocarelli *et al*. (2007) upon serum dioxin levels, quality of semen, and the reproductive hormones of males of different age groups, who all got exposed to dioxin during an accident in Seveso, Italy in 1976. It was concluded from study that the exposure to dioxin decreased sperm quality.

## 11.4 *Effect on epididymis*

Several studies has been done in regards to the impact of dioxin on reproductive system of human but no in vitro studies are there to find the exact impact of dioxins on the epididymis

of human. Although some accidental cases are there which support that dioxin has adverse impact on reproductive system but that can't be considered as the complete significant data to deduce result. However, some studies on rats have been performed as they have physiological similarties with the human to study the determintal impact of dioxin on epididymis. These studies on rat had shown that dioxin have negative effect on androgen, testosterone and dihydrotestosterone (Foster *et al.*, 2011).

## 12 Developmental impact

Developmental effects are the most sensitive health end-point. Children, mainly breastfed infants, are the population most at risk and some of the impacts on them are often irreversible (WHO, 2002; FAO/WHO, 2006). The sources of dioxin in infants/fetus are through the placenta or breastfeeding. Newborns are the most susceptible group as they have fast developing organ systems. Dioxin is also known for crossing the placental barrier and, therefore, it can impact the functions of the trophoblast in the blastocysts and cell-lineage formation and, after that, influence embryonic development in humans (Myatt, 2006). Alterations in proliferation and differentiation of cell may underlie the teratogenic response to this class of chemicals as well.

Studies on animals suggest an association between exposure to dioxin and developmental impact. Developmental exposure to TCDD induces many abnormalities in animals like placental dysfunction in rats (Ishimura *et al*., 2009; Kawakami *et al*., 2006). It adversely affects fetal survival in mice or rats (Vorderstrasse *et al*., 2004; Bell *et al*., 2007). Structural defects following dioxin exposure have been reported, including the malformation of cleft palate and Hydronephrosis in murine (Abbott *et al*., 1987; Peters *et al*., 1999). Dioxin exposure slows brain growth and the neurodevelopment of fetuses or offspring, particularly in male rats (Nishijo *et al*., 2007).

Epidemiologic studies have controversial results, as there are limitations due to small sample size and narrow range of exposure, etc. Still, there are there are some significant reports evidently showing the impact of dioxin exposure on humans. Several reports and case studies have been published on the residents of Seveso, Italy, as they were highly exposed to dioxins. Some reported developmental defects in progeny, while some of them could not observe those results. There was a noticeable elevation in the incidences of congenital disabilities observed in that area after an accident, in comparison to pre-accident levels (Bisanti *et al*., 1980). Another study by Wesselink *et al*. (2014) on the population of Seveso, Italy, followed them for more than 20 years to look for an association between maternal dioxin and developmental impact. There was no accelerated evidence of stillbirth, delay on fetal growth, or effect on gestational length. Ngo *et al*. (2006) reported that parental exposure to the dioxin-containing compound that is Agent Orange could be related to an enhanced congenital disabilities risk.

A report by Wolfe *et al*. (1995) on children of Vietnam veterans who got exposed to Agent Orange in the "Ranch Hand" operation, suggested that there exists a relationship between parental exposure and neural tube defects risk in the progeny of exposed parents. Although the result was not perfect due to limitations of epidemiological studies, there was no significant elevation in risk of stillbirth, birth defect, or delays in development.

A previous report by the Institute of Medicine (US) on the health impact of Agent Orange concluded an increase in spina bifida risk in children of United States soldiers

exposed to Agent Orange during the war in Vietnam (Institute of Medicine, 2003). Another meta-analysis by Anh Duc Ngo *et al*. (2010) reported that paternal exposure to chemical Agent Orange was related to spinal bifida case.

A study on a population of a highly dioxin-contaminated site area around Seveso, Italy, was conducted to research congenital disability (Mastroiacovo *et al*., 1988). Relative risks of congenital disabilities and malformations were calculated, and the data was unable to reveal any enhanced risk of congenital disabilities due to dioxin. However, the number of pregnancies in an exposed population selected for study was not sufficient enough to show a specific increase in teratogenic risk by dioxin exposure (Mastroiacovo *et al*., 1988). Koopman-Esseboom *et al*. (1996) studied several mother-infant pairs to depict the relation between dioxin levels in the mothers and cord blood plasma and its impact on neurological and psychological development. The study revealed that there was a small delay observed in psychomotor development.

## 13 Effect on skin

Skin is the largest sense organ that acts as a barrier from the external environment and protects the body from foreign toxic compounds. Dioxin, a persistent organic pollutant, has a very profound impact on skin physiology, and therefore it is responsible for several skin problems (Bock and Köhle, 2009). Chloracne is the most commonly observed skin problem that occurs after dioxin exposure. Therefore, it is manifested as a "hallmark" of dioxin exposure (Suskind, 1985). Chloracne caused due to mild exposure may disappear after several months, but that caused by high dose exposure or severe cases may persist for more than 30 years (Watanabe *et al*., 1999).

Dioxin elicits several adverse impacts on human skin in addition to chloracne, like edema, erythema, a decrease in secretion of sebum, skin thickening, and pigmentation. Palmoplantar hyperkeratosis and palmoplantar hyperhidrosis of the sweat gland had also been observed during dioxin intoxication (Ju and Zouboulis, 2013). Although dioxin exposure causes several dermal problems, there is still no knowledge of the exact pathways of dioxin which leads to hyperpigmentation or melanogenesis. Aryl hydrocarbon receptor (AHR) is the main receptor where the dioxin binds and some of the studies showed that dioxin exposure to melanocytes leads to the activation of AHR signaling pathways (Luecke *et al*., 2010; Mandal, 2005). This signaling pathway is potentially involved in the pathogenesis of chloracne. High levels of the AHR receptor expression in epidermal cells and epidermal keratinocytes supports the possibility of AHR involvement in the pathogenesis of chloracne (Tang *et al*., 2008; Panteleyev and Bickers, 2006). Several cases reported confirmed the occurrence of chloracne after exposure to dioxin and dioxin-like compounds.

The explosion in herbicide manufacturing plant in 1976 at Seveso, Italy, released more than 30 kg dioxin. The population residing near this area was highly exposed, and several investigations by different researchers were done to study the effect of dioxin on the human population. Hay reported chloracne in the Seveso city of Italy in 1976. A follow-up study performed by Assennato *et al*. (1989) reported the prevalence of chloracne in subjects who got exposed to dioxin at Saveso. Neuberger (1998) studied 159 chloracne cases from 1969–1975 in workers who were involved in the production of herbicides at the plant. In the exposed workers, the frequency of chloracne was highest – that is, it persists in 32% of

the population. The most recent case of dioxin exposure was of the Ukraine president. In the year 2004, the former president of Ukraine, Victor Yushchenko, was poisoned by dioxin, and after two weeks, he developed facial edema and chloracne (Pape and Stahlmann, 2007; Schrenk and Chopra, 2013). A study was done by Suskind (1985) to study the impact of dioxin in causing chloracne. He selected workers involved in making the herbicides 2,4,5-trichlorophenoxyacetic acid and trichlorophenol and these workers were followed for 30 years. Eighty-six percent of the workers who were exposed developed chloracne for some time and 52.7% of them had chloracne 30 years after exposure (Suskind, 1985).

## 14 Mortality and cancer

Till now, none of the studies has reported acute instances of death in exposed populations by high concentrations of dioxin. Several epidemiological studies investigated mortality in populations exposed to dioxin or dioxin-related chemicals. Many of them observed no significant enhancement in death incidence in people who experienced occupational exposure due to handling chemicals containing dioxins (Ott *et al*., 1980; Zack and Suskind, 1980) or in workers who were exposed to dioxin because of an accident at BASF AG facility in Germany (Ott and Zober, 1996). A report by Fingerhut *et al*. (1991) observed an enhanced risk of mortality due to cancer in workers handling chlorophenol and phenoxy herbicides. Bertazzi *et al*. (1997), after 15 years of follow-up of the highly exposed population at Seveso, Italy, reported no increases in mortality numbers. On the other hand, a recent study by Consonni *et al*. (2008) observed an increase in mortality after 25 years. They also confirmed toxic and cancer risks due to exposure by these chemicals. Collins *et al*. (2009) examined several workers in Midland, Michigan, who were exposed to dioxins during trichlorophenol production to study the impact of exposure on mortality. No particular trend in mortality was noticed.

Cancer is one of the significant causes of death across the globe (Torre *et al.*, 2015). It has been speculated that the mortality due to cancer will increase to 11.4 million by 2030 (Jemal, 2010). The exact cause behind this is still a puzzle, but external as well as internal factors are the contributors behind this. Eighty percent of cancer is mainly caused due to environmental factors (Higginson and Muir, 1977). Dioxin or TCDD is a widespread environmental contaminant which has been declared as a potent carcinogen (Travis, 1991; Knerr *et al*., 2006). Several studies have been done on dioxin exposure impact and they interpreted that this increases the risk of human cancer (Birnbaum, 1994; Beebe *et al*., 1995). Studies also proved the increased risk of lymphoma and sarcoma in a population exposed to dioxin released from incinerators (Floret *et al*., 2003; Viel *et al*., 2000). Several case-controls and cohorts have been carried out to study the carcinogenicity of dioxin. A major weakness of these epidemiological studies is the lack of proper exposure data and information about concomitant exposure to other compounds. Epidemiological studies proved dioxin was a potent carcinogen, and they also indicate human carcinogenicity at multiple sites (Table 6.1). Both *in-vivo* and *in-vitro* experiments proved dioxin was a potent cancer-causing substance (Schaufler *et al*., 2002; Schecter, 2013). Carcinogenicity of dioxin is initiated by binding to the AHR receptor, which subsequently leads to changes in cell replication, proliferation, gene expression, and apoptosis and later on by this way, it increases the occurrence of cancer in human (Baan *et al*., 2009). Manuwald (2012) follow up a dioxin-exposed population for 23 years

*Table 6.1* List of studies on different types of cancer incidence in several dioxin-exposed locations.

| *Location* | *Type of cancer* | *Reference* |
|---|---|---|
| Seveso, Italy | Breast cancer | Warner *et al*., 2002 |
| Seveso, Italy | Prostate cancer | Consonni *et al*., 2008 |
| Seveso, Italy | Lymphatic & hematopoietic tissue neoplasms | Pesatori *et al*., 2009 |
| Midland, Michigan | Multiple cancers (non-Hodgkin's lymphoma and leukemia, Soft tissue sarcoma, prostate) | Collins *et al*., 2009 |
| New Zealand | Lung, mouth, liver, prostate, kidney, brain cancer and non-Hodgkin's Lymphoma | Mannetje, 2005 |
| Vietnam | Prostate cancer | Schecter *et al*., 2009 |
| Hamburg, Germany | Respiratory, esophagus, bladder, kidney, and rectum cancers | Manuwald *et al*., 2012. |
| Ludwigshafen, Germany. | Respiratory and digestive cancer | Ott and Zober, 1996 |
| Dutch | Urinary and genital cancers | Boers *et al*., 2010 |
| Besançon | Breast cancer in females | Viel *et al*., 2008 |
| Audeux, Besançon | non-Hodgkin's Lymphoma | Floret *et al*., 2003 |
| Venice, Italy | Sarcoma | Zambon *et al*., 2007 |

after people were exposed to dioxin in a chemical plant at Hamburg Germany to study the long-term effect with regard to mortality due to cancer. Increase in mortality due to cancer in men as well as women was observed. A retrospective cohort study was carried out by Boers *et al*. (2010). In two of the chlorophenoxy manufacturing factories, an increased risk for all cancer was observed, and a very slight enhancement in cancer mortality risk by cancer was noticed. The most crucial evidence for studying the impact of dioxin is the Seveso, Italy, population. An increased risk of breast cancer among women exposed to dioxin during the environmental explosion was observed (Warner *et al*., 2002).

## 15 Cardiovascular hepatic and neural effect

Exposure to dioxin is related to ischemic heart disease (IHD) and all other cardiovascular diseases (CVD). Several studies have shown the positive correlation between them, but a few findings have not observed any association between them. Increased risk of mortality from IHD as well as all CVD also has been found in exposed populations. Ha *et al*. (2007) studied an association between serum concentration and dioxins using the National Health and Nutrition Examination Survey (NHANES) data. They reported a positive correlation between the prevalence of CVD and dioxin concentration. Bertazzi *et al*. (1998), in his study on the long-term impact of dioxin exposure in the population in the Seveso accident, reported a significant enhancement in deaths from cardiovascular disease. They also found increased mortality in males due to CVD 15 years after the incident. Flesch-Janys *et al*. (1995) carried out a retrospective cohort study on workers from an herbicide producing plant in Hamburg, Federal Republic of Germany. Associations between cardiovascular diseases, ischemic heart diseases, mortality, and exposure to dioxins were investigated and from that a robust dose-dependent relation between them could be predicted. Humblet *et al*. (2008) concluded that there exists a significant and consistent correlation between IHD and CVD mortality and dioxin dose. Calvert *et al*. (1998) studied workers dealing with 2,4,5-trichlorophenol or its derivatives in United States chemical plants for more than 15 years to find an association between dioxin exposure and cardiovascular disease.

The workers were having significantly high serum concentration levels but not any significant relationship was observed between them.

Hepatic toxicity is reported in acute as well as chronic exposure to dioxin. Short-term exposure to high levels impairs the liver function (Marinković *et al*., 2010). Half of the patients with chloracne suffered from hyperlipemia, hypercholesterolemia, and hyperphospholipidemia. Various studies have found a relationship between dioxin and liver cancer in mammals (Fingerhut *et al*., 1991). A marginal increase in chronic liver disease was observed in a subgroup in a follow-up study of employees working at BASF who were exposed to dioxin after a chemical reactor incident of 1953 (Zober *et al*., 1994). Mocarelli *et al*. (1986) studied 1500 children from the exposed population of Seveso, Italy. after the environmental disaster to find whether lipid metabolism and hepatic functions were impacted by dioxin exposure or not. Sixty-nine children showed enhanced levels of alanine aminotransferase and y-glutamyltranspeptidase activities in 1976–1977 but that was in reference limit and vanished with time.

It has been reported at several places that dioxin exposure alters the normal functioning of nervous systems (Fisher, 1999; Jones and De Voogt, 1999; Kampa and Castanas, 2008). The environmental disaster that occurred due to explosion polluted the environment of Seveso, with a high concentration of dioxin impacting the population as they showed a higher incidence of the deleterious impact on (PNS) peripheral nervous system (Bonaccorsi *et al*., 1978). Barbieri *et al*., 1988 investigated 152 people from Seveso suffering from chloracne to study the dioxin impact on PNS. They observed that peripheral neuropathy was not apparent in any of patients suffering from chloracne.

## 16 Conclusion

"Dioxins," persistent organic pollutants, are the primary pollutants of our ecosystem as they are frequently added in the soil, water, and air. They are a critical outcome of a human-made industrial revolution and pollution. The environment receives dioxin pollution from cement industries, combustion industries, smoke, domestic waste, etc. Exposure can occur via different sources, like environmental, accidental, industrial, and residential. The human population mainly gets exposed to this persistent organic pollutant through air, soil, water, and living biota. These contaminants enter into the body mainly through food and some via dermal or respiratory paths. Dioxin tends to bioaccumulate in the body as it have a longer half-life, hence can remain in the human body for many years. These pollutants also reach to non-target organisms, like aquatic organisms, plants, and animals and, through food chain, enter into humans. After entering into humans through different environmental routes, it activates specific metabolic pathways via particular matrices. Human cells have specific hydrocarbon receptors, AHR, which deliver false signaling when the particular kind of dioxin ligand binds to it, resulting in the over expression or wrong expression of genetic and metabolic signaling reactions. Exposures of humans to dioxins have negative impacts on their bodies, and dioxins are a causative agent of many problems. Regarding human health, dioxins show acute and chronic exposure, which results in multiple diseases like chloroacne, nausea, vomiting, edema, erythema, hepatic damage, nervous system damage, and cardiovascular damage. Dioxins are also potent carcinogens, which can cause several types of cancer. The rate of occurrence of cancer gets enhanced in areas having high levels of dioxin; several

case studies have proved it. Dioxins also have a negative impact on the human immune system as they decrease leukocytes, lymphocytes, and immunoglobulins. It also impacts the reproductive and endocrine systems. Exposure to dioxin in increase the incidence of endometriosis and also disturbed the estrogenic balance thus impact the fertility. Epididymis, sperm quality, and sperm count also decrease. Teratogenic effects also are observed as a result of dioxin exposure during early stages of the developmental process. More studies on these kinds of persistent organic pollutants call for resisting future environmental and human health problems. Industrialization is necessary but over manufacturing can inhibit the human race.

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Chapter 7

# The epigenetic effects of dioxins

*Simone O. Heyliger and Marilyn D. Saulsbury*

## 1 What are dioxins?

Dioxins are structurally diverse polyhalogenated aromatic hydrocarbons that include polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) (Rysavy *et al*., 2013). The PCDDs include 75 individual compounds and PCDFs include 135 compounds (US EPA, 2006). These compounds are highly toxic and are major environmental contaminants and pollutants. Because dioxins are resistant to high temperatures and chemical degradation (oxidation and hydrolysis), they have long half-lives and thus persist in the environment for very long periods of time. The sources of dioxins are multivariate and can include both natural and industrial sources. Moreover, dioxin releases from a source can be in the form of products, air emissions, water discharges, and solid residues (US EPA, 2006).

PCDDs and PCDFs are produced as by-products of high temperature sources (including cement kilns, asphalt plants, and carbon reactivation furnaces) and of industrial activities such as waste incineration fuel combustions, secondary copper recovery, metal (aluminum and iron) smelting, synthesis of certain herbicides (e.g. Agent Orange), and the bleaching of wood pulp during the manufacturing of paper (Hutzinger *et al*., 1985; Arisawa *et al*., 2005; US EPA, 2006; Cagnetta *et al*., 2016). Natural sources of PCDDs and PCDFs include volcanoes and forest fires (US EPA, 2006; Cagnetta *et al*., 2016). Unlike PCDDs and PCDFs, which are produced primarily by unintentional sources, PCBs are mixtures of approximately 209 synthetic chlorinated biphenyl compounds (ATSDR, 2000). Of the 209 PCB congeners, only 12 are thought to have dioxin-like toxicity and thus are classified as dioxin-like PCBs (dl-PCBs). PCBs were once used as plasticizers and in the production of electrical components, thermal insulation material, adhesives, and tapes. Though PCBs have been banned since 1976, they are still present in older electrical equipment such as transformers, capacitors, voltage regulators, and electromagnets (ATSDR, 2000).

Because of their hydrophobic nature and resistance towards environmental degradation, these chemicals primarily accumulate in the fatty tissues of animals and humans (US EPA, 2006). Plants contain very small amounts of PCDDs. However, spinach and several leafy vegetables may contain dioxins at much higher concentrations (Schecter *et al*., 1997; Amakura *et al*., 2003). Hence, human exposure to dioxins and dioxin-like compounds occur predominantly through the consumption of dioxin-contaminated meat, milk, fish, and poultry (Schecter *et al*., 1994). PCB exposure, in particular, is most likely to occur from the consumption of sportfish obtained from contaminated bodies of freshwater. Individuals living near superfund/hazardous waste sites may be exposed to PCBs via inhalation

of PCB contaminated air or via ingestion of water from contaminated water tables (ATSDR, 2000). Minor exposure to PCDDs, PCDFs, and PCBs may occur through inhalation of cigarette smoke (Muto and Takizawa, 1989). However, it should be noted that the minute amounts of dioxin and dioxin-like compounds in tobacco smoke are sufficient to activate dioxin response elements (DRE) and to produce biological effects (Lofroth and Zebuhr, 1992; Kasai *et al*., 2006).

## 2 Mechanism of action of dioxins

The most studied Dioxin is 2,3,7,8-tetrachlorodibenzo-p-dioxin, which is often referred to as "dioxin." 2,3,7,8-tetrachlorodibenzo-p-dioxin is considered the most toxic and is used as the reference standard for analyzing the biological activities of dioxin-like compounds. Presumably, the mechanisms underlying TCDD and dioxin-like toxicity is due to its interactions and activation of the aryl hydrocarbon receptor (AHR), which is a ligand dependent basic-helix loop-helix transcription factor Ma & Whitlock (1997). In its inactive form, AHR is part of a multiprotein complex consisting of heat shock proteins 90 (HSP 90), co-chaperone protein p23, and the aryl hydrocarbon receptor-interacting protein (AIP), also known as ARA9 and XAP2 Ma & Whitlock (1997). Once TCDD binds to the AHR, the receptor undergoes conformational changes and translocates to the nucleus. From there, the AHR combines with aryl hydrocarbon receptor nuclear translocator (ARNT) to initiate transcription of target genes with promoters containing the Dioxin Response Elements (DREs) or Xenobiotic Response Elements (XREs). In addition, the AHR/ARNT transcriptional complex recruits other proteins (e.g., SRC-1, CBP, NCoA2) that also modulate transcriptional activity and chromatin structure (Beischlag *et al*., 2008; Marshall and Kerkvliet, 2010). AHR/ARNT target genes include xenobiotic metabolizing enzymes, such as members of the cytochrome P450 enzymes (CYP1A1, CYP1A2, *and* CYP1B1), aldehyde dehydrogenase, and glutathione-S-transferase. Moreover, AHR may enhance gene expression of proteins which regulate cellular growth and differentiation through transactivation of the Epidermal Growth Factor Receptor (EGFR) directly via the up-regulation of EGF-like proteins or through AHR-induced increases in cAMP (Haarmann-Stemmann *et al*., 2009).

## 3 Human health effects of dioxins

The health consequences that occur subsequent to exposure to dioxins are multivariate. Chronic exposure to dioxins and dioxin-like compounds are linked to increased risk or incidences of cancers (Chen *et al*., 2014), immune system impairment (Ahrenhoerster *et al*., 2014), endocrine disruption, developmental delays, as well as reproductive and cardiovascular dysfunction in human, cellular, and animal models (Anwar and Lehmann, 2014; Ott and Zober, 1996; Chen, 2013; Wikenheiser *et al*., 2013; Zhang *et al*., 2013).

### 3.1 *Cancer and dioxins*

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF), and 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) have been designated as human carcinogens (IARC, 2012). In the landmark study of German men accidentally exposed to TCDD in 1953, researchers found that workers who received the highest

doses were 50% more likely to develop gastrointestinal cancers (Ott and Zober, 1996). A study of Korean veterans of the Vietnam war (Yi and Ohrr, 2014) found that exposure to the herbicide Agent Orange (which contained high levels of TCDD) increased the incidence of cancers in this population. Exposure to Agent Orange was also associated with higher incidences of mouth and throat cancers and was associated with increased risk of gastrointestinal (GI) cancers; particularly of the stomach and the small intestines (Yi and Ohrr, 2014).

### 3.2 *Cardiovascular disease and dioxins*

In addition to cancer, several studies indicate that exposure to PCDDs, PCDFs, and PCBs, or to TCDD-contaminated Agent Orange is associated with cardiovascular diseases and that the dose required to produce cardiotoxicity may be lower than the TCDD burden required to induce cancers (Flesch-Janys *et al*., 1995; Humblet *et al*., 2008; Puga, 2011; Yi *et al*., 2013).

### 3.3 *Dioxins, endocrine disruption, and reproductive dysfunction*

Dioxins, through their interactions with the AHR, interfere with numerous hormone systems. In animal models, dioxins are associate with reproductive dysfunction. TCDD produces dose-dependent increases in endometriosis in murine, rat, and primate models and may even lead to reduced fertility in females through the loss of progesterone receptor responsiveness (Cummings *et al*., 1996; Yang *et al*., 2000; Bruner-Tran *et al*., 2010). Similar results have also been observed in human cellular and tissue models. Moreover, the exposure to TCDD leads is associated with transgenerational (F1–4) endometriosis in mice (Bruner-Tran *et al*., 2018). Given that other endocrine disruptors such as methoxychlor and vinclozolin induced an adult phenotype of decreased spermatogenic capacity (cell number and viability) and increased the incidence of male infertility (Anway *et al*., 2005) as well as kidney disease, ovary disease, and obesity in both male and female mice F1-F3 generations (Mannikam *et al*., 2014a) through epigenetic mechanisms, it possible that TCDDs and dioxin-like compounds are utilizing similar mechanisms. Indeed, a recent study suggested that TCDD exposure may cause transgenerational inheritance of male infertility epigenetic alterations (Manikkam *et al*., 2012b).

### 3.4 *Developmental delays and neuropsychological impairments*

Mounting evidence indicate that dioxins and dioxin-like compounds can induce neurological changes in the developing fetus and infant (Jacobson and Jacobson, 1996; Stewart *et al*., 2008; Tai *et al*., 2011). PCDDs, PCDFs, and PCBs can be transferred to the fetus via the placenta or to the infant via the ingestion of breastmilk (Chen *et al*., 2001; Wittsiepe *et al*., 2007; Tai *et al*., 2011). Moreover, prenatal exposure to polychlorinated biphenyls (PCBs) leads to delays in cognitive development in children (Jacobson and Jacobson, 1996; Stewart *et al*., 2008). A longitudinal (Lake Michigan) study of 212 children exposed *in utero* to PCBs were found to be associated with lower full-scale and verbal IQ scores, with the highest exposure dose group (> 1.25 μg/g fat) exhibiting the largest decrease (6.2 points) in IQ scores in comparison to other exposure and aged match control groups (Jacobson and Jacobson, 1996). A more recent study, developed to replicate the Lake Michigan findings, found that children exposed to PCBs also scored three points

lower on the full scale IQ test and scored lower on the Freedom from Distractibility tests (Stewart *et al*., 2008).

### 3.5 *Immune suppression and dioxin*

Toxicologists have long recognized that TCDD and dioxin-like PCBs haves a suppressive effect on the immune system. TCDD has been shown to cause thymic atrophy decreased host resistance to pathogens and tumors, suppressed fetal lymphocyte development and maturation, and suppressed adaptive immune responses, including antibody production, cytotoxic T lymphocyte (CTL) activity, and alterations in hypersensitivity reactions in animal models (Kerkvliet, 2002; Lawrence and Kerkvliet, 2006; Marshall and Kerkvliet, 2010; Shimada *et al*., 2015). However, human studies are extremely limited, and the results have been somewhat mixed. A recent study of adult females suggests that dioxin-like PCBs exposure is not associated with immunosuppression (Spector *et al*., 2014), whereas several studies indicate that in utero exposure to dioxin-like PCBs is associated with smaller thymus glands and higher risks of upper respiratory tract infections (Park *et al*., 2008; Stølevik *et al*., 2011; Jusko *et al*., 2012). One study suggested that in utero exposure to TCDD leads to gender-specific changes in the transcriptional profile of genes involved in modulation of the immune system (Hochstenbach *et al*., 2012). Interestingly, this study also noted that there were alterations in the acetylation status of histone H4-K12 in males; suggesting that the epigenetic mechanism may play a key role in the transcriptional regulation of immune system genes (Hochstenbach *et al*., 2012).

## 4 Environmental epigenetics and dioxins

Environmental epigenetics is a relatively new area of discipline and represents a new approach for conducting risk assessments (Ray *et al*., 2014). To date, most focus studies have centered around the epigenetics of heavy metals such as arsenic, mercury, lead, and calcium. Epigenetics refers to inheritable modifications of gene expression that are not resultant from changes in DNA sequence. Epigenetic regulation occurs primarily via three major mechanisms: histone modifications, DNA modifications, and the production of small non-coding RNAs. Histone modifications include acetylation, methylation, phosphorylation, ubiquitination, and sumoylation; whereas, DNA modifications are characterized by methylation while non-coding RNAs take the forms of microRNAs (miRNA) and small inhibitory RNAs (siRNA). Though there is a preponderance of information regarding the effects of dioxin on signaling pathways and gene-pathways, very little information exists regarding its ability to alter gene expression through epigenetic mechanisms. However, AHR is able to activate the EGFR signaling system which, in turn, alters DNA methylation. Moreover, TCDD activation of AHR leads to increases in CREB levels. CREB then activates p300/CBP, which has intrinsic Histone Acetyl transferase (HAT) activity (Yildirim *et al.*, 2014). Hence, theoretically, TCDD can alter gene expression via epigenetic mechanisms.

### 4.1 *Dioxin and histone modifications*

Histones are basic proteins that bind to DNA to form nucleosomes and ultimately chromatins. The basic nucleosome consists of five types of histones H1, H2A, H2B, H3, and H4.

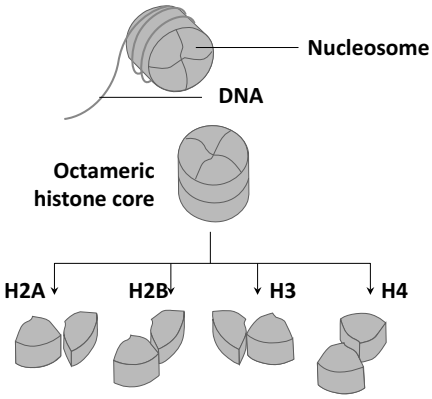


*Figure 7.1* Individual core histone monomers (H2A, H2B, H3 and H4) combine to form octameric structures

Histones H2A, H2B, H3, and H4 form an octamer consisting of two molecules each which contain lysine rich tails that are the sites for acetylation, methylation, and ubiquitination. Other histone amino acids subject to chemical modifications (methylation and phosphorylation) include threonine, serine, and arginine. Theoretically, histone modifications can alter electrostatic charges and recruit binding proteins that are frequently part of chromatin remodeling complexes, thereby making the chromatin more or less accessible for other proteins, such as enzymes and transcription factors (Smits *et al*., 2014).

***Histone Acetylation and Deacetylation:*** Histone acetylation, by way of the actions of histone acetyltransferase (HAT), attenuates the positive charges on histones, thereby reducing their affinity for the negatively charged phosphate groups of DNA backbone, leading to the "relaxing" of the chromatin structure (euchromatin) and to increased transcription due to easier accessibility to the DNA. Conversely, deacetylation through the actions of histone deacetylase (HDAC), reverses the process by increasing the positive charges and enhancing the binding of the histones to DNA to produce densely packed, closed chromatin conformation (heterochromatin).

Numerous studies have shown that AHR and its ligands, such as dioxin and benzo[a] pyrene, are capable of inducing and repressing the expression of numerous genes. The alterations in gene expression are associated with changes in cellular growth, differentiation, and functions. Since histone modifications regulate gene expression, it is feasible that TCDD toxicity and AHR actions may be mediated through the alteration of histone acetylation status. Several studies suggest that dioxin induces CYP gene expression through histone modifications. Indeed, Schnekenburger and colleagues (2007), demonstrated that AHR activation was associated hyperacetylation of histone H3K14 and H4K16, trimethylation of histone H3K4, and phosphorylation of H3S10 subunits associated with the promoter region of *CYP1A1* gene. In addition, dioxin stimulation of AHR leads to recruitment of the histone acetylase (HAT) coactivators p300 and steroid receptor coactivator 2 to the promoter regions of *CYP1A1* and *CYP1B1* (Beedanagari *et al*., 2010). Moreover, these epigenetic changes in in the CYP 1 genes were shown to be consistent regardless of the ligand that activates the AHR-ANRT complex (Ovesen *et al*., 2011).

Dioxin may also exert gene silencing activities through histone modifications. Papoutsis *et al*. (2010) reported that TCDD increases the association of mono-methylated-H3K9, DNA-methyltransferase-1 (DNMT1), and methyl-binding domain protein-2 with the BRCA-1 promoter and caused a reduction in BRCA-1 expression. Takeda and colleagues (2012) reported that TCDD induces histone deacetylases (HDACs), specifically HDAC 1, 5, and 7 in the rat fetuses of mothers exposed to the environmental pollutant. Moreover, the elevation of HDAC 1, 5, and 7 activities were associated with the silencing of the fetal expression of the beta subunit of luteinizing hormone (LHβ) as well as gonadal steroidogenic proteins and gonadotropin related signaling pathways.

### 4.2 *Dioxin and DNA methylation*

In mammalian systems, the addition or removal of methyl groups to highly enriched areas of genomic DNA containing cytosine residues located next to guanines (so called CpG islands) can alter gene expression. Methylation of these cytosine residues to create 5-methylcytosine in promoter regions occurs primarily through the activity of DNA methyl transferases (DNMT) and is generally associated with gene silencing (Suzuki and Bird, 2008). Presumably methylation can inhibit gene expression by reducing the ability of transcription factor interaction with cognate *cis* elements and by promoting the binding of methyl-CpG-binding proteins (McClure *et al*., 2011). The methyl-CpG-binding proteins (MBPs) silence transcription by facilitating the development of condensed chromatin, thereby preventing the binding of transcription factors (Klose and Bird, 2006; McClure *et al*., 2011). Conversely, removal of the methyl functional groups is more likely to lead to gene expression.

The importance of DNA methylation status had been demonstrated repeatedly by numerous labs which have linked several diseases (cancer, diabetes,) to altered methylation/demethylation. By far, cancer remains the most studied disease state with regards to altered methylation status whereby hypermethylation (gene silencing) of promoters noted in various tumor suppressor genes and checkpoint regulators have been observed in numerous cancer cells. Given that TCDD is implicated in cancer pathogenesis and has been shown to modulate the levels of proteins which modulate cellular proliferation and apoptosis, it is plausible that TCDD mediates its activities via alteration in methylation status. Indeed, a study of 1016 senior-citizens (age 70 or above) from Sweden revealed that exposure to dioxin congener octachlorodibenzo-p-dioxin (OCDD) was associated with global DNA hypermethylation (Lind *et al*., 2013) mediated through the AHR. McClure and colleagues (2011) reported that treatment of C57BL/6 mice with 3 μg/kg TCDD resulted in the altered methylation in 43 genomic regions (RAMs), of which 56% (24) were annotated to genes: 54% (7/13) of hyper-, 58% (7/12) of hypo-, and 56% (10/18) of new methylations in splenocytes. Conversely, Bastos Sales *et al*. (2013) demonstrated that TCDD causes global hypomethylation in murine 3T3-L1 preadipocytes but had little effect on the methylation status of human neuroblastoma cells, thereby suggesting that DNA methylation may be tissue specific or limited to a discrete number of genes in select cells (Bastos Sales *et al*., 2013).

TCDD epigenetic changes may have permanent effects, which can be passed onto subsequent generations (imprinting). Several reports have cataloged higher incidences of congenital defects and reproductive abnormalities in both children exposed *in utero* and in children of parents exposed to TCDD (Le and Johansson, 2001). TCDD administration

in rodents during pregnancy decreased the sperm concentration and the prostate weight of the male offspring (Foster *et al*., 2010; Manikkam *et al*., 2012; Somm *et al*., 2013). Since TCDD is generally accepted as having minimal genotoxic and clastogenic activity, it is likely that TCDD causes its reproductive and teratogenic effects through the alteration of DNA methylation status in germlines. In fact, Manikkam *et al*. (2012) reported that ancestral exposure of a gestating female to dioxin promotes an altered fetal gonadal development and epigenetic reprogramming of the male germline. The genes differentially methylated in the F3 generation encoded various transcription factors, growth factors, signaling pathways, and hormones with the most notable affected genes being luteinizing hormone beta (LHB), Src homology 2 domain containing transforming protein 2 (SHC2), neuroblastoma ras oncogene (NRas), transforming growth factor beta (TGFbi), and breast cancer anti-estrogen resistance 3 (BRCA3). Given that these genes are implicated in cancer and reproductive dysfunction, this data suggest that TCDD may be exerting its endocrine disrupting effects through the alteration of signaling pathways activated by key hormones/growth factors as well as transcription factors and oncogenes.

The revelation that TCDD can alter global methylation status engenders potential questions related to how dioxin promotes tumorigenesis. One of the key hallmarks of carcinogenesis is aberrant hypermethylation of tumor suppressor genes (Feinberg and Tycko, 2004) and not surprisingly, recent studies suggest that TCDD may also increase the methylation of suppressor genes. Ray and Swanson (2004) reported that TCDD represses the expression of the tumor suppressor proteins $p16^{INK4a}$ and p53 in human keratinocytes. Moreover, the TCDD-induced repressed expression of p53 was completely reversed by DNA methyltransferase inhibitor 5-azacytidine (5-aza-dC); whereas, TCDD induced suppression of $p16^{INK4a}$ was partially reversed by 5-aza-dC. In addition, methylation specific PCR confirmed that $p16^{INK4a}$ promoter region was indeed hypermethylated subsequent to TCDD treatment (Paajarvi *et al.*, 2005).

TCDD effects may also extend to BRCA-I, a tumor suppressor gene which is integral for the maintenance of genomic stability (repair of DNA damage) and cellular growth. Mutations in BRCA-1 lead to diminished BRCA-1 function and are associated with higher incidences of breast and ovarian cancer. Moreover, hypermethylation of BRCA-I promoter is more likely to be associated with higher tumor grades, cancer invasiveness, ER negative, and triple negative breast cancer characteristics (Esteller *et al*., 2000; Bal *et al*., 2012; Jacot *et al*., 2013); thereby suggesting that repression of BRCA-1 may portend chemoresistance or even poorer prognosis for patient survival. The relationship between TCDD and BRCA-1 is complex and may actually be part of an elaborate network whereby AHR and BRCA-1 interact which each other to maintain tight control over hormone mediated and xenobiotic response pathways via the regulation of phase I and II metabolizing enzymes as well as possibly cell survival genes. For instance, TCDD represses E2-dependent activation of BRCA-1 transcription (Hockings *et al*., 2006; Zhao and Ramaswamy, 2014). Conversely, BRCA is capable of facilitating the AHR-ANRT-mediated transcription. Papoutsis *et al.* (2012) reported that TCDD exposure causes AHR dependent hypermethylation of the BRCA-1 promoter by the recruitment of DNA methyl transferases (DNMT1, DNMT3a, and DNMT3b). Moreover, this hypermethylation was associated with reduced expression of BRCA1 and was reversed by the administration of AHR antagonist resveratrol. This data suggests a role for TCDD in breast cancer tumorigenesis and further indicates that the AHR may represent a possible target for reducing sporadic breast cancer tumorigenesis in vulnerable populations (Papoutsis *et al*., 2012).

### 4.3 *Dioxin and miRNA Regulation*

MicroRNAs (miRNA) are a class of noncoding RNAs of 19–24 nucleotides in length that bind the 3′-untranslated regions (3′UTR) of the messenger RNA (mRNA) molecule. The genes encoding the miRNAs are located in the introns of protein-coding genes, or in both introns and exons of non-coding transcripts. Most miRNA genes are transcribed by RNA Polymerase II to generate primary transcripts, which are then processed to smaller approximately 70-nucleotide hairpin looped precursor molecules (pre-miRNAs), which in turn are cleaved by the Dicer enzyme to produce the miRNAs (Tan *et al*., 2014). When bound, the miRNA induces the degradation or inhibition of translation of mRNA, thereby leading to the silencing or partial silencing of the target gene. The effects of miRNA functions are multivariate and thus these non-coding RNAs have been implicated in the mediation of cellular differentiation (Cheng *et al*., 2009), growth and proliferation (Ivey and Srivastava, 2010; Delaloy *et al*., 2010; Niu *et al*., 2013), as well as metabolism and death. Moreover, dysregulation of miRNA has been linked to cancer (Volinia *et al*., 2006; Duan *et al*., 2011; Liu *et al*., 2013), atherosclerosis (Ruiz and Chakrabarti, 2013), diabetes (Kong *et al*., 2011; Tyagi *et al*., 2011), and Alzheimers (Banzhaf-Strathmann *et al*., 2014; Liu *et al*., 2014).

The study of the ability of dioxin to regulate miRNA is in its infancy and, thus, there is limited knowledge regarding which miRNA is activated or repressed by TCDD. However, a recent study of the role of AHR in leukemogenesis (Rager and Fry, 2012) suggest that the AHR may also exert effects through miRNA epigenetics mechanisms. Gordon *et al.* (2014) report that the AHR receptor activated by polyaromatic hydrocarbons is able to mediate the expression of several miRNAs species (miR-25, miR-15a, miR-16, miR-92, miR-125b, miR-141, and miR-200a) with TCDD specifically inducing the expression of miR-25 and miR-92. Both miR-92 and miR-25 target the p53 gene (Gordon *et al*., 2014). Interestingly, TCDD has been recently shown to suppress p53 expression (Reyes-Hernández *et al*., 2010; Ambolet-Camoit *et al*., 2010)

Notably miR-25 overexpression is associated with gastric cancer proliferation and metastases (Li *et al*., 2015; Zhao *et al*., 2014), ovarian cancer (Wang *et al*., 2014), esophageal carcinoma (Wu *et al*., 2014), colorectal cancer (Li *et al*., 2014), and lung cancer. In addition, elevated levels of miR-25 are associated with poorer prognostics and outcomes as it relates to cancer. There are several hundred genes which could be potential targets of miR-25. Targets for miR-25 that are of particular interest include the transducer of ERBB2 (TOB1), which serves as a putative tumor suppressor and regulator of the cell cycle, *Wwp2*, *Adam23,* and *Rab8b* as well as regulators of autophagy ULK1 and BECN1 (Wang, 2014; Lu *et al*., 2012). Interestingly, dioxin has been recently shown to modulate autophagy (Fiorito *et al*., 2011) and, hence, alteration of miR-25 levels represents a means by which dioxin can mediate apoptosis and cell cycling processes (Wang *et al.*, 2014).

The miR-92 belongs to the miR-17–92 cluster, which is also known as Oncomir-1 because members of this family have been found to be dysregulated in numerous cancers. The miR-17–92 cluster is thought to modulate a variety of cellular processes, including cell growth, cell differentiation, apoptosis, cell motility, cell adhesion, cell invasion, and angiogenesis. Other members of this cluster include miR-17, miR-18a, miR-19a, miR-19b, and miR-20a. Micro-RNA 92 may regulate approximately 100 genes and has been suggested to serve as a proto-oncogene (Zhao *et al*., 2013). Among the possible targets for miR-92 (Mavrakis *et al*., 2011; Zhao *et al*., 2013) is the tumor suppressor

gene PTEN (phosphatase and tensin homolog). This gene is primarily responsible for attenuating the effects of phosphatidylinositol 3,4,5-trisphosphate through dephosphorylation, which leads to the suppression of PI3K/Akt signaling pathway. The PI3K/Akt is a strong mediator of cancer cell survival as is activated by several cytokines and growth factors (Marte and Downward, 1997; Chan *et al*., 1999; Li *et al*., 2013). In addition, overexpression of and mutations of PI3K/Akt pathway have been linked to chemoresistance (Ghebeh *et al*., 2014; O'Brien *et al*., 2014), cancer pathogenesis (Song *et al*., 2014), and to the development of aggressive forms of cancers (Russo *et al*., 2014; Maemura *et al*., 2014). Presumably, PI3K/Akt activates anti-apoptotic proteins such as Bcl-2. Conversely, Akt inhibitors have been shown to induce apoptosis and reverse the effects of chemoresistance in certain cancer cells (O'Brien *et al*., 2014). Therefore, miR-92 suppression of PTEN gene product may ultimately lead to the overactivation of Akt activity, enhanced proliferation, and enhanced cancer cell survival. It should be noted that the AHR and TCDD has been shown to stimulate the PI3K/Akt pathway (Tannheimer *et al*., 1998; Davis *et al*., 2001). Indeed, TCDD inhibits apoptosis and enhances cellular proliferation through PI3K/Akt dependent mechanisms (Tannheimer *et al*., 1998; Davis *et al*., 2001; Davis *et al*., 2003; Xu *et al*., 2014). Moreover, PI3K/Akt inhibitors reversed TCDD proliferative effects of NNK-induced tumors in A/J mice (Chen *et al*., 2014). Taken collectively, these preliminary studies suggest that TCDD may promote tumor promotion and survival by increasing the expression of miRNA that negatively regulates tumor suppressor genes. The relationship between TCDD and miR-92 represents an exciting new line of study since it offers a viable explanation as to how TCDD and the AHR modulate PI3K/Akt and, thus, is capable of promoting tumor progression and survival.

Though the preponderance evidence suggests that TCDD's role in cancer biology is to facilitate tumor formation, numerous laboratories reported that TCDD and similar polyaromatic hydrocarbons may also exhibit anti-metastatic properties in breast cancers. Several studies indicate that TCDD can inhibit invasiveness and colony formation in human (SKBR3 and MDA-MB-231 cells) and rodent breast cancer cell models (Holcomb and Safe, 1994; Hall *et al*., 2010; Wang *et al*., 2014). Using a miR array as a screening tool, Zhang and colleagues (2012) identified microRNA-335 (miR-335) as an AHR-responsive miR induced by TCDD in ER negative MDA-MB-231 and BT474 cells. Interestingly, miR-335 has been demonstrated to possess anti-metastatic properties (Tavazoie *et al*., 2008) and to repress cellular differentiation (Samaraweera *et al*., 2014). In addition, miR-335 regulates the formin protein family, which is responsible for modulating actin restructuring activities during cellular migration and differentiation (Lynch *et al*., 2013). Moreover, overexpression of this microRNA correlated to enhanced metastasis-free survival of patients with primary breast cancer (Tavazoie *et al*., 2008). Zhang and colleagues (2012) further demonstrated that induction of miR-335 correlated with reduced expression of SOX4, a transcription factor that mediates embryonic development and cellular migration (Wang *et al*., 2014). However, administration of siAHR reversed the attenuating effects of TCDD on SOX4 mRNA and protein expression, while overexpression of SOX4 partially blocked TCDD mediated inhibition of cellular migration and invasion in ER negative MDA-MB-231 cells. This data seemingly contradicts studies which demonstrate that TCDD interferes with the activities of tumor suppression. However, the competing actions of TCDD as both tumor promoter and as an anti-metastatic agent raises the intriguing theory that hormonally driven, environmental induced cancers may be the result of the dysregulation of AHR-ANRT transcription pathways.

## 5 Conclusion

The induction of different epigenetic mechanisms in various tissues or different cell types may offer insight into complex nature of dioxin toxicity and AHR functions. Though dioxin is considered tumorigenic, it seems able to arrest certain types of metastatic cancers. This contradictory property is very similar to the observation made with certain SERMs such as tamoxifen, which can reduce breast cancer proliferation while having a promoting effect on uterine cancers. Nonetheless, a better understanding of the role of dioxin in modulating gene expression through changes in epigenetic status represents new opportunities for developing therapeutic interventions, which can more accurately target pathways involved in metastasis and carcinogenesis.

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Chapter 8

# Dioxins and cancer

## Detailed insight into the mechanism

*Gulnaz Javan, Sheree Finley, Ashim Malhotra and Shivani Soni*

## 1 Introduction

### 1.1 *Chemistry and toxicological effects*

Rapid industrialization for the last two centuries has revolutionized and mechanized our day to day living. On the flip side, it has led to many challenges, such as environmental pollution and related health hazards. Dioxins and dioxin-like compounds are members of the polyhalogented aromatic hydrocarbons, formed as industrial by-products, that represent major environmental contaminants. Dioxins are diverse in their structure, including polychlorinated dibenzodioxins (PCCDs), polychlorinated biphenyls (PCBs), and dibenzofurans (PCDFs) (Rysavy *et al*., 2013). Dioxins structurally are characterized by two benzene rings connected by two oxygen atoms and can have four to eight chlorines forming considerably diverse congeners. Out of 210 possible congeners, only 17 have toxicological importance due to substitution at the 2,3,7, and 8 positions, resulting in preventing or slowing down their conversion to polar metabolites by phase I and phase II enzymes (Nebert *et al*., 2004).

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most potent and toxic congener responsible for inducing liver damage, wasting syndrome, dermal toxicity, reproductive and developmental toxicity, immunotoxicity, and carcinogenicity (Bertazzi *et al*., 2001). Dioxins are lipophilic compounds and, thus, can diffuse freely across the cell membrane, resulting in their accumulation in lipids by simple partitioning in tissues and leading to slow elimination in feces (Sorg *et al*., 2009). These lipophilic pollutants are mainly present in the nonpolar sediment fractions of aquatic ecosystems, especially near industrial and harbor areas. Dioxins recognized as persistent organic pollutants (POPs) entail aquatic food chains, including mollusks, fish, birds, and mammals, ultimately leading to human exposure (Montano *et al*., 2013). Acute lethal poisoning gives a better perspective of the outcome of common, long-term cumulative exposure of dioxins. Although no lethal dose has been known in humans, few cases of significantly high level of exposures have been reported, such as Ukraine President Victor Yushchenko's severe poisoning in September 2004 (Sorg *et al*., 2009), and the October 1997 case of TCDD poisoning of five textile plant workers in Vienna, Austria (Rysavy *et al*., 2013). Cases in which large populations were exposed to TCDD have also been reported in the past, such as an explosion of a chemical plant in Seveso, Italy, in 1976 (Mocarelli *et al*., 1996), human population exposed to dioxin-contaminated rice oil in Japan and Thailand (ECS SCF, 2000), and U.S. veterans exposed to Agent Orange during the Vietnam War (Sinks, 2014).

### 1.2 *Dioxin sources and bioaccumulation*

Dioxins are considered to be aerially emitted from uncontrolled municipal and hazardous waste incinerators. Certain manufacturing processes, such as chlorine bleaching of paper pulp, herbicide and pesticide production, metal refineries, emissions from automobiles using leaded fuels, hospital incinerators, and even the backyard burning of trash are culprits of dioxin contamination (Fries, 1995). Since D=dioxins are by-products of combustion, natural processes such as forest fires also contribute significantly to environmental contamination (Gribble, 1994).

Attributed to their high physical, chemical, and biological resistance, their breakdown by ultraviolet radiation and biodegradation is very slow. Furthermore, because of their hydrophobicity, they tend to bioaccumulate in organic material, especially lipids and thus primarily fatty tissues in animals (Linden *et al*., 2010). Since dioxins are released into the air as a consequence of industrial processes and settle on plants and enter into soil and water, it makes its way into the food chain of humans. As mentioned above, food sources account for more than 90% of dioxin exposure to humans, especially with the consumption of commercially made animal products such as dairy, meat, and human milk with high fat content (Lorber *et al*., 2009).

### 1.3 *Aryl hydrocarbon receptor targets for dioxins*

Poland *et al*. (1973) were the first to hypothesize that an intracellular binding protein or receptor may be the primary site of binding for TCDD and other related dioxins (Poland and Glover, 1973). Later, their group purified and characterized the mouse hepatic AHR protein (Poland *et al*., 1976). Toxicological and other cellular effects of dioxins are mediated by binding to the AHR receptor protein. AHR is a ligand activated transcription factor, i.e. as soon as TCDD or other ligands bind to AHR, it translocates from the cytoplasm to the nucleus of the cell. In the cytosol, AHR is bound to a molecular chaperon complex containing two heat shock protein (HSP-90) molecules, co-chaperone p23, immunophilin-like AHR interacting protein (AIP/ XAP2/ ARA9) (Barouki *et al*., 2007), and tyrosine kinase c-src (Enan and Matsumura, 1996) to prevent unnecessary nuclear translocation of unliganded AHR. Upon ligand binding, AHR undergoes a change in the confirmation leading to dissociation of the chaperon complex. Once the receptor translocates to the nucleus, it forms a heterodimer with the AHR nuclear translocator (ARNT) protein, which interacts with dioxin response elements (DRE), also known as xenobiotic response elements (XRE) present on Ah-responsive gene promoters to activate transcription of a battery of dioxin inducible genes that catalyze conjugation and the metabolisms of xenobiotics (Kennedy *et al*., 2014).

The human AHR gene encodes for a 96 kDa protein containing 848 amino acids (Dolwick *et al*., 1993). AHR contains two structural motifs (Fuji-Kuriyama and Kawajiri, 2010): the basic helix loop helix (bHLH) and PER-ANT-SIM (PAS) domain, which is characteristic of a particular superfamily of transcription factors to which ARNT and AHR repressor (AHRR) are also associated (Abel and Haarmann-Stemmann, 2010). The N-terminal bHLH domain is essential for DNA binding and protein dimerization, while the PAS domain is required as a docking site for other PAS proteins and molecular chaperone HSP-90 (Gu *et al*., 2000). The PAS domain is also known to function as a binding target for small molecule ligands, including environmental contaminants such as TCDD (Burbach *et al*., 1992). The C-terminal is housed by a glutamine-rich transactivation domain (TAD), which interacts with basal transcription factors TATA binding proteins

(TBP), RAP30, and RAP74 subunits of transcription factor IIF (TFIIF). These factors play a significant role in target gene activation. The association of TBP and AHR suggests that AHR plays an important role in the initiation of transcription by recruiting TBP to that TATA element and later stabilization of the TBP/TATA complex. TFIIF is responsible for binding the polymerase enzymes to specific promoter sequences of interest during initiation of transcription, and it is also considered to be involved in the elongation process of transcription as suggested by its association with RNA polymerase II in the elongation complex (Rowland *et al*., 1996).

Once the AHR/ARNT heterodimer binds to XRE (Fuji-Kuriyama and Kawajiri, 2010), it induces chromatin remodeling and recruiting various transcriptional regulators, such as CREB binding protein (CBP/P300), steroid receptor coactivator 1 (src-1), steroid receptor coactivator 2 (src-2), nuclear receptor co-activator 2 (NCoA2), nuclear receptor co-activator 3 (NCoA3), receptor-interacting protein-140, Brahma-related gene 1 (BRG-1), thyroid hormone receptor/retinoblastoma interacting protein 230 (TRIP230), silencing mediator for retinoic acid and thyroid hormone receptors (SMRT), GRIP1, p-TEFβ are required depending on the cell type. TRAP/DRIP/ARC complex with RNA polymerase II are recruited at the promoter region of gene CYP1A1 to launch mRNA transcription (Fuji-Kuriyama and Kawajiri, 2010). Phase I and phase II drug metabolizing enzymes primarily monooxygenase of CYP family (CYP1A1, CYP1A2, CYP1B1) in the liver are considered targets for dioxin-activated AHR (Bock and Kohle, 2006). Other enzymes activated by dioxin-induced AHR are CYP2S1, CYP2A5, ALDH3, GSTA1, UGT1A1, UGT1A6, UGT1A7 and NQO1 (Linden *et al*., 2010). Finally, dioxin-induced AHR signaling pathway is turned off by a two-step event: nuclear export of liganded AHR and cytoplasmic proteolysis by ubiquitin mediated 26S proteasome complex (Davarinos and Pollenz, 1999).

Further studies using *AHR*-/- mice have shown that the physiological function of AHR receptors is not limited to mediating effects of xenobiotic mice (McMillan and Bradfield, 2007). *AHR*-/- mice exhibit reduced fertility, structural and functional abnormalities in various tissues, i.e. failure of developmental closure of ductus venosus in the liver, vascular abnormalities in cardiovascular organs, reduced number of mature follicles due to anomalies in the reproductive tract, uric acid stones in the urinary bladder, anomalies in stem cells and their functions, as well as tissue specific immunomodulatory effects (Safe *et al*., 2013). Under normal physiological conditions, AHR plays an important role in cell proliferation, invertebrate development, cell adhesion and migration, reproduction, tumor suppression, as well immunity and autoimmunity (Barouki *et al*., 2007; Aguilera-Montilla *et al*., 2013). AHR and its ligands are responsible for significant interference in signaling cascades involving cytokines and growth factors such as EGFR, TGFβ, and TNFα (Haarmann-Stemmann *et al*., 2009), tumor suppressor proteins such as retinoblastoma (Rb) protein (Elferink *et al*., 2001), stress responsive genes such as NF-kB and p38-MAPK (Puga *et al*., 2000; Korashy *et al*., 2011), contact inhibition involving JunD pathway, cell-cell adhesion involving JNK pathways (Dietrich and Kaina, 2010), cell cycle and cell proliferation such as AKT-ERK pathways (Chen *et al*., 2014), and apoptosis signaling pathways, i.e. those related to p53 (Paajavri *et al*., 2005).

### 1.4 *Non-carcinogenic effects of dioxins*

Lethality after exposure to dioxins and $LD_{50}$ doses showed considerable sensitivity variation in different species. Although much of the progress has been done in the pathology related to dioxin exposure in animals, the human difference in toxicology profiles that is

typically observed in pre-clinical models like mice and rats has yet to be explored. Animal toxicology often does not correlate with human toxicology profiles as observed in clinical trials. Dioxins have a plethora of toxic responses and pathophysiology involving various tissues. Studies related to toxicological effects of dioxin give us insight into deregulated physiological functions on exposure to xenobiotics and species-specific sensitivity variation in toxic responses. Skin toxicity known as chloracne is the most consistent pathology related to dioxin toxicity, characterized by hyperkeratotic skin disorders affecting hair follicles, sebaceous glands, and interfollicular dermis (Bock and Kohle, 2006). Various studies have revealed that dioxins can be reprotoxic and act as well as teratogens (Peterson *et al*., 1993). The effect of TCDD on the cleft palate in mice is well studied and it is observed that the cleft is formed with growing palatal shelves that fail to fuse. In contrast, human embryonic palatal shelves are affected in a similar way but at relatively higher concentration ($10^{-8}$ M) than murine ($10^{-10}$ M), suggesting less sensitivity in humans than mice (Abbott and Birnbaum, 1991). Following mothers exposed to dioxin-contaminated rice oil in the Yusho and Yu-cheng incidents in Japan and Taiwan, respectively, the children born had low birth weight, developmental delays, behavioral disorders, hearing loss, and effects on sexual development (Larsen, 2006). Moreover, neurodevelopmental delays, neurobehavioral effects, and thyroid hormone alterations have been reported in several studies from the USA and Europe (ECS SCF, 2000; Montano *et al*., 2013). Furthermore, the role of reactive oxygen species (ROS) in dioxin-induced neuronal toxicity has been well studied, suggesting TCDD is responsible for the inhibition of human neuronal cell proliferation and peripheral nerve damage (Mates *et al*., 2010). Additionally, exposure to dioxin leads to significant reduction in feeding and, as a result, decline in body weight also known as wasting syndrome in laboratory animals (Pohjanvirta *et al*., 1994).

The liver, adipose tissues, and lungs are considered to be primary sites of AHR ligands deposition in humans. The respiratory system has been known to be sensitive to dioxin exposure, resulting in chronic bronchitis, allergic asthma, chronic obstructive pulmonary disease (COPD,) and lung cancer (Chiba *et al*., 2011). Dioxin has immunomodulatory effects; B and T cells and consequently host resistance to infections are significantly affected (Boverhof *et al*., 2004). AHR and its ligands play an important role in the regulation of Treg (T-cells) and TH17 cell differentiation. The relationship between Treg and TH17 cells is critical for the onset of autoimmune disease and also has been suggested in autoimmune encephalomyelitis (EAE) in murine models (Veldhoen *et al*., 2008).

## 2 Dioxins as carcinogens

According to the American Cancer Society, cancer is the leading cause of death in developed nations and the second leading cause in economically developing nations. Studies show that the number of cancer-related deaths is expected to increase to 11.4 million worldwide by 2030 (Jemal *et al*., 2010). Environmental pollutants such as dioxins are considered to be major culprits in the development of cancer (Tsay *et al*., 2013). Environmental factors such as xenobiotics interacting with humans account for two thirds of cancer cases in the United States (Sankpal *et al*., 2012). The dioxin TCDD is classified as a Group I carcinogen by the International Agency of Research in Cancer (IARC) and is considered to be a non-mutagenic and non-genotoxic carcinogen operating as a potent promoter and weak initiator. TCDD acts as a carcinogen by modifying the signaling pathways and by indirect oxidative

damage to DNA resulting in tumor promotion (Knerr and Schrenk, 2006). Moreover, ligand-activated AHR has been suggested to play a role in tumor progression from benign to malignant stages, in addition to initiation and promotion, in animal models as well as humans (Dietrich and Kaina, 2010). Additionally, certain studies have shown TCDD inhibits cell senescence, which can be an important aspect of mechanisms involved for TCDD to act as a carcinogen (Ray and Swanson, 2009). The following sections will give detailed insights into carcinogenicity of dioxins, leading to various types of cancers and the mechanism involved.

### 2.1 *Dioxins and lung cancer*

Lung cancer is caused by exposure to environmental pollutants like dioxins, polycyclic aromatic pollutants, formaldehyde, benzene, acrolein, and cigarette smoke. Some of these environmental pollutants are ligands, which directly bind to AHR. AHR activation causes histone modification, activation of xenobiotic metabolism, and tumorigenesis (Chiba *et al*., 2011; Wong *et al*., 2010). The five-year survival rate of lung cancer is about 15%. Surgical resection of the lungs in the early stages of lung cancer has a high cure rate. This gives scientists hope to find markers for early detection and, therefore, a high cure rate for the cancer. Chronic inflammation and the lack of the ability to repair epithelial cells of small and central airways are the most common mechanisms of lung carcinogenesis. While most carcinogens exert their effects by binding to distal bronchoalveolar epithelial cells, some directly bind to AHR, which in turn results in tumorigenesis, cell proliferation, the lack of cell to cell adhesion, and inflammation and formation of DNA elements. Since cigarette smoking activates AHR, it is one of the major causes of lung cancer (Tsay *et al*., 2013).

Numerous studies have shown TCDD toxicity to cause lung cancer. At high levels, TCDD toxicity causes COPD and lung cancer in humans (Kogevinas, 2000). TCDD is a tumor promoter not a mutagen. It does not directly interact with DNA (Knerr and Schrenk, 2006). Nevertheless, the mechanism of action for TCDD is not clear. It is suggested that protection of MCF-10A cells from apoptosis by activation of anti-apoptotic kinase B and also ERK pathways are how it aids cancerous cells (Davis *et al*., 2000; Davis *et al*., 2001). Therefore, TCDD's tumor-promoting effect is the cause of its thermo-carcinogenicity (Chen *et al*., 2014).

Studies also contribute some non-cancerous effects to dioxin exposure. Some of these effects include diabetes, cardiovascular effects such as IHD, and reproductive effects such as sex ratio (male:female) gender effects (Mocarelli *et al*., 1996).

### 2.2 *Dioxins and breast cancer*

Breast cancer ranks as the number one malignancy among women in Western Europe and the United States of America effecting about 150,000 women in United States and 500,000 worldwide. Age, family history, and country of birth are the major risk factors listed for breast cancer. However, despite advances in science and enhanced screening, breast cancer rates have increased (White *et al*., 1990). This situation suggests possible environmental factors being involved in the process of carcinogenesis. There are limited explicit links associating breast cancer risk and environmental contaminants (Wolff and Wesyon, 1997). The two major environmental risks that are well defined in increasing breast

cancer are exposure to radiation and alcohol consumption (Wolff and Wesyon, 1997). Even though diet is considered to increase the risk of breast cancer in developed countries, its role is not clear (Wolff and Wesyon, 1997). Exposure to DDT and other chemical emissions are also considered as carcinogenic. Dioxins are among the most studied chemicals for their carcinogenic effects.

Dioxin is a six-member heterocyclic ring where oxygen replaces the two carbon atoms in the ring. TCDD is an odorless and colorless solid at room temperature. It is a by-product of burning organic materials or organic synthesis. It is used in Agent Orange and is a persistent contaminant of the environment. TCDD carcinogenicity has been a subject of extensive studies (Safe *et al*., 2013). In 1997, the IARC classified TCDD as a group I human carcinogen. It is believed that dioxin-like compounds produce their toxicity by acting on a specific AHR. AHR is present in large numbers in multiple organs and tissues (Cole *et al*., 2003). It is also associated with different types of cancer cells, and studies have shown that inhibition of AHR receptors retard and decrease proliferation and/or invasion of cancerous cells (Safe *et al*., 2013). Kociba *et al*. reported TCDD effects on breast cancer for the first time in 1978 where female Sprague Dawley Rats were given TCDD in their diet for two years. The result of the study suggested that inhibition of 17β-estradiol by AHR activation via TCDD caused reduction of breast cancer in the rats (Kociba *et al*., 1978). However, the carcinogenicity of TCDD is dependent on the model and timing of exposure. Brown *et al*. showed that exposure to only a 1 μg/kg of TCDD during the prenatal period can cause higher carcinogen-induced mammary tumor formation (Brown *et al*., 1998). There are two main pathways for TCDD's anti-estrogenic activity. Both pathways exert their effects by depletion of estrogen receptor α (ERα) and estrogen 2 receptor (E2). Spink *et al*. found that increased oxidative metabolism of E2 as a result of induction of CYPIA1/CYP1B1 is one of the pathways, whereas Wormke *et al*. listed regulation of ERα via liganded AHR activation of proteasomes as the other pathway for TCDD's anti-estrogenic/carcinogenic effect (Wormke, Castro-Rivera *et al*., 2000). Survival rates of hormone dependent (ER-positive) and hormone independent (ER- and PR-negative) breast cancer patients have direct relationship with AHR expression. Higher AHR expressions are associated with elevated overall survival rate and increased relapse time for these patients (Wormke, Castro-Rivera *et al*., 2000; Wormke, Toner *et al*., 2000). One *in vitro* study showed that the proton pump inhibitor Prilosec (Omeprazole) can reduce the rate of breast cancer invasion. It was also noted that Prilosec reduced the rate of metastasis to the lung by decreasing expression of two pro-metastatic genes, matrix metallopeptidase 9 (*MMP-9*) and C-X-C motif chemokine receptor 4 (*CXCR4*) (Wong *et al*., 2010). The effect of Omeprazole is AHR dependent and exerts its effects by down-regulating the *CXCR4* gene (Jin *et al*., 2014). This study opens the door to use AHR active medicines such as Prilosec in late stage breast cancer treatments; however, more studies are ongoing, and the outcome of these studies will dictate if expanding the use of Prilosec alone or in combination with chemotherapy agents in combating breast cancer is warranted (Luciani *et al*., 2004).

### 2.3 *Dioxins and multiple myeloma*

Multiple myeloma (MM), which constitutes nearly 10% of all hematologic cancers, is a malignant disorder of single clone of plasma cells derived from B cells (Kyle *et al*., 2004). Clinical symptoms vary, e.g. unexplained back pain, failure in renal functions,

being prone to bacterial infections, hyper-viscosity, anemia, amyloidosis, and hypercalcemia (Smith *et al*., 2006). The malignancy occurs in bone marrow and neighboring bones are usually attacked by the malignant cells leading to disarrangement of skeletal structures, which causes pain and fractures (Kyle and Rajkumar, 2009). Plasma cells can also infiltrate to multiple organs, which explains the variety of symptoms. Monoclonal protein is produced in excessive amounts by the plasma cell clone (Kyle and Rajkumar, 2009). Monoclonal proteins not only result in hyper-viscosity in blood but they also interfere with renal function (Smith *et al*., 2006). The age of the patients is an important factor for deciding the treatment options, including high-dose therapy (HDT) stem cell transplantation (Smith *et al*., 2006) and immunotherapy. Almost 60% of the patients are under 60 and only 2% are less than 40 years old. The patients between 60 and 65 are also around 15% of the diagnoses. The upper age limit of the intensive therapy has increased, and the preferred therapy is still oral chemotherapy. MM is observed in males a little bit more than it is observed in females, while it is observed two times more in African American descendants than Caucasians (Kyle *et al*., 2004).

Multiple processes are thought to contribute to malignant transformation of MM. Mutations are accumulated in single clone plasma cells and they transform into "monoclonal gammopathy of undetermined significance" (MGUS), which is an asymptomatic premalignant stage of the MM (Hideshima *et al*., 2004; Kyle *et al*., 2006). Some patients also develop a more advanced but still asymptomatic intermediate stage known as "smoldering multiple myeloma" (SMM) (Fonseca *et al*., 2004). Translocations including chromosome 14 are common in MM and constitutes more than 50% of all medullary MM cases (Kawano *et al*., 1988). Those translocations put the tightly regulated proto-oncogenes, such as Cyclin D1 (11q13), fibroblast growth factor receptor 3 (4p16), and C-maf (16q23) under the direction of Ig heavy chain enhancer (14q32), which increases the expressions of the oncogenes (Bezieau *et al*., 2001). Translocations including t(4;14), t(14;16) and deletions or monosomy of chromosome 13 are considered as bad severe prognoses (Bezieau *et al*., 2001). In addition to translocations, activating mutations of *N*- and *K-ras* genes are observed in 35–50% of MM patients (Bommert *et al*., 2006). These mutations are rare in MGUS patients, which indicates that those mutations are secondary events in transformations (Bommert *et al*., 2006). Besides genetic events, the transformation is also assisted by bone marrow microenvironment (BMM), which releases factors, i.e. interleukin 6 (IL-6) and provides a suitable environment for tumors to grow (Catlett-Falcone *et al*., 1999). IL-6 induces the JAK/STAT pathway and prevents apoptosis of the cells (Kawano *et al*., 1988; Stuhmer *et al*., 2005). Interestingly, *p53*, which is one of the most common genes that is mutated in all cancer types, is rarely mutated and provides an excellent therapeutic target for MM treatment (Avet-Loiseau *et al*., 1999; Wang *et al*., 2014).

Although MM has a genetic background, it can be triggered by chemicals such as dioxins and dioxin-like compounds (Dannenberger *et al*., 1997). They can either be emitted as a by-product of certain chemical production or accumulate as a result of incomplete combustion (Kim *et al*., 2005; Bertazzi *et al*., 1999). Many studies have assessed the dioxin exposure and MM development risk (Mannetje *et al*., 2005; Bhattacharyya *et al*., 1995). Several studies after the disaster in Seveso, Italy, showed that the exposure increased the rate of hematologic malignancies, including Non-Hodgkin's lymphoma (NHL), MM, and leukemia (Mannetje *et al*., 2005). Animal studies also demonstrated that lymphatic tissues are primarily targeted by dioxins (Mannetje *et al*., 2005). Dioxins also

trigger CYP1B1 enzymes through AHR (Shimada *et al*., 2001; Shimada *et al*., 1999). The CYP1B1 enzyme activates several carcinogens, which increased the risk of a variety of cancers, including MM (Hayes *et al*., 1996; Aravalli *et al*., 2013). Another group investigated the exposure of farmers and workers to phenoxy herbicides and dioxin in New Zealand in an epidemiological study (Bhattacharyya *et al*., 1995). They found a correlation with MM and exposure to those chemicals, but the molecular details were not investigated.

### 2.4 *Dioxins and hepato-pancreato-billary cancer*

Primary liver cancer types consist of hepatoblastoma, hepatocellular carcinoma (HCC), angiosarcoma, and cholangiocarcinoma (CCA) (Nordenstedt *et al*., 2010). HCC is the most common liver cancer type that is observed in 80% of whole liver cancer cases (Nordenstedt *et al*., 2010). While HCC is the fifth most common cancer among all cancer types, it is the third deadliest cancer around the world (Yang *et al*., 2011). The molecular and histo-pathological background of HCC is not well understood. The current understanding of HCC initiation suggests that the malignant transformation requires the accumulation of mutations and cooperation of the microenvironment of the transformed cells (Aravalli *et al*., 2008). The target of the mutations involves critical signaling pathways where the proto-oncogenes, *B-catenin*, *Axin1*, *PI-3-kinase*, and *K-ras* are activated, while the tumor suppressor genes, including *p53*, *Rb1*, *CDKN2A*, *IGF2R*, and *PTEN* were deactivated (Hoyert *et al*., 2006). HCC is usually triggered by an underlying liver disease, which is frequently cirrhosis (Nordenstedt *et al*., 2010). The variety of signaling pathways and the diversity of affected proto-oncogenes and tumor suppressor genes involved in HCC indicate that HCC is a heterogeneous cancer with plenty of risk factors, including exposure to hepatitis viruses, vinyl chloride, tobacco, foodstuffs contaminated with aflatoxin B1 (AFB1), heavy alcohol intake, nonalcoholic fatty liver disease, diabetes, obesity, diet, coffee, oral contraceptives, and hemochromatosis (Hoyert *et al*., 2006).

Pancreatic cancer is a highly lethal disease, and each year 100,000 patients are diagnosed with pancreatic cancer around the world and only less than 3% of those patients could survive five more years (Efthimiou *et al*., 2001). It is ranked the fourth most common cause of cancer death (Efthimiou *et al*., 2001). Medical and surgical therapies have shown considerable advances over the last several decades; however, that progress still is not good enough to improve mortality rates (Pesatori *et al.*, 2003). In addition to medical and surgical advancements, the molecular background of pancreatic cancers has also been investigated. Despite extensive studies, the molecular basis is still full of unanswered questions. Several genetic alterations in both tumor suppressors (CDKN2A, SMAD4, and TP53) and oncogene (KRAS) have been reported so far (Tuveson and Hingorani, 2005; Jaffee *et al*., 2002; Maitra and Hruban, 2008; Anderson *et al*., 1996; Peller *et al.*, 2003). Epidemiologic studies revealed several risk factors, but the most prominent ones are old age, smoking, family history, and occupational exposure (Li *et al*., 2004). It has been observed more commonly in males and African American populations (Kylie *et al.*, 2004).

## Acknowledgements

This work was partially supported by National Science Foundation (NSF) grant HRD 1401075.

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Chapter 9

# Ecological risk of dioxin exposure

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## 1 Introduction

Dioxin has a chemical name 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD), which belongs to a chemical group called persistent organic pollutants (POPs). Dioxin is a broad term that comprises polyhalogenated dibenzo-p-dioxins and furans – compounds which are the by-products of combustion and industrial processes and also of polyhalogenated biphenyls, naphthalenes, and azo/azoxybenzenes (White *et al*., 2009). Dioxin and its related compounds are toxic in nature and the main source is industrial pollutants. These industrial contaminants are ubiquitous and unrelenting in the environment. There are different anthropogenic and natural sources of dioxin which contaminate the environment and consequently enter into the food chain. Dioxins can enter our environment by both natural as well as anthropogenic sources. The natural sources comprise volcanic eruptions and forest fire, whereas the anthropogenic sources include industrial processes, smelting, and manufacturing of pesticide and herbicides. Among the wastes, dioxin pollution is mainly the result of the incomplete combustion of hospital waste and solid waste. The food, soils, and sediments are reported to have highest amount of dioxin and air, water plants have the lowest levels (Fries, 1995; Alcock and Jones, K., 1996). Among the sources of dioxin pollution, municipal waste, sewage sludge, and medical waste incinerators top the chart, along with the burning of residential and industrial wood (Kampa and Castanas, 2008; Kulkarni *et al*., 2008). Therefore, today's unrestrained incineration of sewage waste is of great concern (McKay, 2002).

Dioxin presents in air, water, and soil. It is absorbed by plants and animals directly through skin via process of respiration or it may choose another path along the food chain. Animals accumulate dioxin mainly through ingestion of contaminated vegetation, water, and soil. Dioxin gets introduced into infants through contaminated mother's milk (Fürst, 2006). The half-life of dioxin in the body is 7–11 years, due to its stability and lipophilic nature, which means they can be absorbed by the fat tissues and can be stored there for a long time (Baughman and Meselson, 1973). Then dioxins can enter the food chain and can lead to biomagnifications. Dioxins are a threat to human and wildlife as they are capable of inducing toxicity.

Dioxin is persistent, toxic, and gets accumulated in the adipose tissues; therefore, it should be regulated and monitored according to environmental policies. The Environment Protection Agency (USEPA, 2004) estimates that dioxin exposure mostly takes place through the diet, also a little exposure takes place from air and skin (USEPA, 2004; Kampa and Castanas, 2008). Therefore, the major sources of dioxin and related compounds

in the human diet are the types of foods. Food types which contain high amounts of fats also have higher dioxin concentrations and these are generally animal products like meat, poultry, eggs, fish, animal fats, and dairy products (Eduljee and Gair, 1996). Green vegetables, leafy vegetables, fruits, and grains are foods with minuscule dioxin concentrations. Due to its lipophilic nature and long half-life, it is persistent and does not break down easily so it gets accumulated in animals (Fries, 1995). Due to persistency, it is bioaccumulative in nature and transported to different organisms at different levels of the food chain; therefore, it is harmful to ecological health. Due to its adverse impact on health, it has drawn the attention of scholars and researchers.

This review will be concentrated on the fate of dioxin in the environment, how it gets transferred to different tropic levels, and its impact on organisms.

### 1.1 *Dioxin in food and its environmental fate*

Dioxin, when released into the environment, binds to incinerator ash, can be protected from degradation by light, and can be suspended for long periods of time before it settles in the soil (IARC, 1997).The half-life varies in the soil surface and subsurface; in the soil surface, half-life is 9 to 15 years, whereas in the subsurface that is below 0.1 cm, it is about 25–100 years. In water, it accumulates in rivers, oceans, and sediments in lakes. Dioxin, due to its hydrophobic and lipophilic properties, can accumulate in fish rather than remaining in water bodies (EPA, 2006). Dioxin can get accumulated readily in fish and gets concentrated in the apex of the food chain. Its concentration is $10^5$ times higher than the surroundings (TSD, 1999). Dioxin can get accumulated in crops and vegetables. Animals such as buffalos, goats, chicken, ducks, etc. have dioxin accumulated in their meat (Schecter *et al*., 2003). Dioxin in food is contributed mainly by meat and dairy products along with beef, eggs, poultry, and pork.

Dioxin absorption is based on the molecular size, solubility, and route of administration (IARC, 1997). About 90% absorption of dioxin occurs via the lungs and small intestine, (Nessel *et al*., 1992) and only 1% absorption is through the skin (IARC, 1997). An investigation that was done on individuals of age group 24 to 81 years showed that almost 87% of the dioxin is absorbed by the gastrointestinal tract. After absorption, the dioxin enters the bloodstream and gets distributed in all organs (Schlummer *et al*., 1998). After entering the bloodstream, dioxin remains for a very short time in blood, which can be attributed to its hydrophobic nature, and gets accumulated in liver and fatty tissues (Ryan *et al*.,1985). The dioxin level is more in meat than in lipids (Schecter *et al*., 2003; Bernard *et al*., 2002). Dioxin is excreted as a water-soluble compound through metabolization and nontoxic compounds in the liver. Excretion rates vary in different species. Dioxin is persistent and can resist degradation by any means viz. physical, chemical, or biological and remains in the environment for many years, and gets accumulated in the environment leading to its biomagnification in the aquatic as well as terrestrial ecosystem (Hatfield, 1998).

### 1.2 *Bioaccumulation of dioxin*

The accumulation of the pollutants present in the environment by the organisms via ingestion, inhalation, and absorption is called as bioaccumulation. Bioaccumulation of dioxin in animals is caused by environmental exposures to these persistent organic pollutants, which originate mainly from soil, air, and water, then transferred to the plants. Therefore, the

environmental concentration and quality play a relevant role in determining bioaccumulation and ensuing transfer to animal products (Smith *et al*., 2007).

Dioxins have a tendency to get attracted towards the fatty and oily compounds than they can dissolve in water; hence, they are more prevalent in animals with body fat. Dioxins have the tendency to be transported long distances through the atmospheric circulation to the polar regions. This transport is because of the properties of volatilization and condensation of the dioxin (Eitzer and Hites, 1989; Corsolini *et al*., 2002). Warm air currents rise, the particles evaporate and travel towards the cooler climates. Salmon, cod, seals, whales, and polar bears have fats which help provide insulation from the low temperatures, and thus, there is more accumulation of POPs in the animals in the colder regions. In the last three decades, the dioxin level has been found to decline in United States due to the reduction in anthropogenic sources. The degradation of the pollutant is very slow, which makes it persistent in the environment even after the ban of the pollutant. The dioxin level in our body should continually decline; levels will be elevated for many years as the dioxin from the past releases persists significantly in the environment (EPA, 2000).

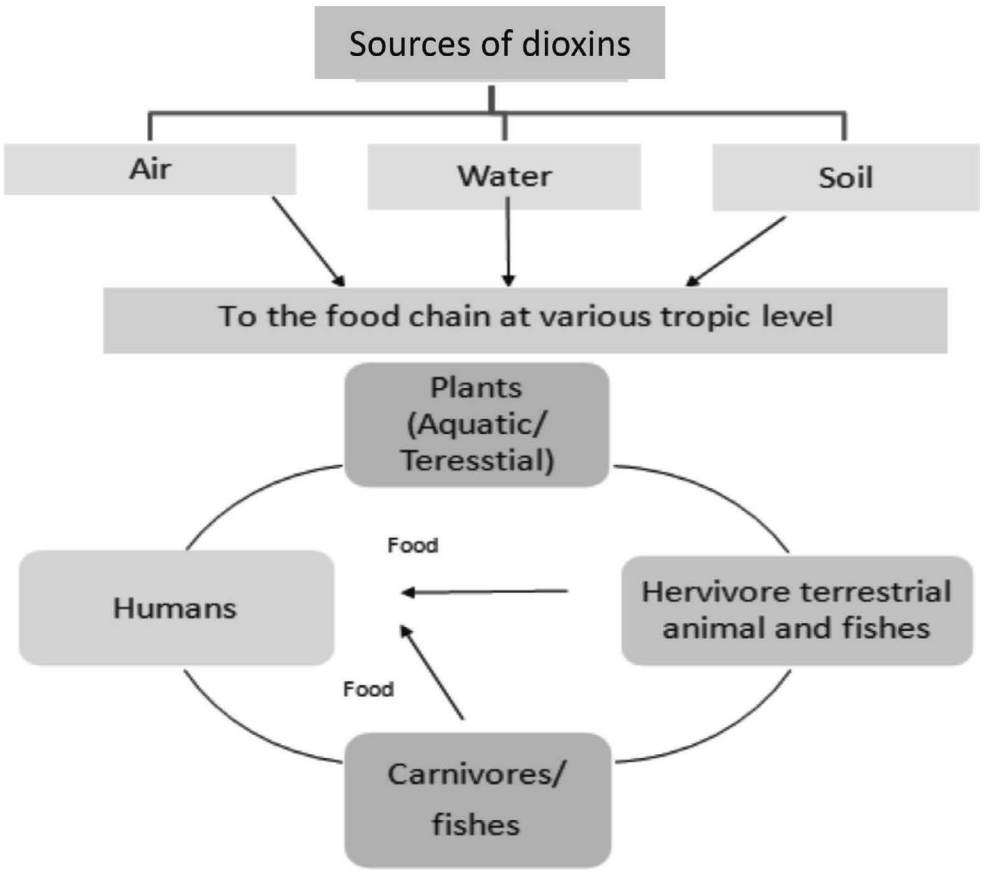


*Figure 9.1* Transfer pathway of dioxin.

### 1.3 *Dioxin toxicity in animals*

In general, the dioxin effect is mediated via aryl hydrocarbon receptors, which is thought to be conserved throughout species. The route of exposure has a limited influence on the effects of the pollutant. Acute or chronic exposure both have similar effects involving development related effects, toxicity of the immune system, and enzyme induction. The response of dioxin toxicity is directly proportional to its tissue concentration (Kogevinas, 2001)

Dioxins are extremely toxic to animals and can lead to the alteration of fundamental development of the cells. According to the EPA, 2000, dioxins potentially can be carcinogenic, teratogenic, and can be endocrine disruptors. The acute effects are immediate and are more dangerous than the chronic effects. Among animals and children, they are affected more by exposure to dioxin, the reason being attributed to their fast growth rate (Lundqvist *et al*., 2006; EPA, 2000). According to the reports by EPA, there is a greater risk to immune system on being exposed to the low levels of dioxin for longer time or high level of dioxin at sensitive time (EPA, 2000).

TCDD is the root cause of wide range of effects in laboratory animals (IARC, 1997; Bimbaun and Tuomisto, 2000). For fetotoxicity, these differences are almost same in species and much less in comparison to adults. Humans are also prone to the adverse effects of the pollutants as depicted by the knowledge of mechanism of action. The major target of the dioxin is the endocrine system in experimental animals, with many hormones being affected. Endocrine effects are also observed in experimental animals due to exposure to dioxin (Foster *et al*., 2009; Kogevinas, 2011).

In both the male and female reproductive systems of monkeys and rats, the steroidogenesis is affected (Dibartolomeis *et al*., 1987; Baldridge *et al*., 2015). In both high and low doses, the reproductive effect is seen in animals. Studies also revealed that exposure to dioxin leads to decrease in sperm count and endometriosis in rats and rhesus monkeys (Birnbaum and Cummings, 2002).

Table 9.2 gives an insight into the reproductive effects observed in animals

In order to study the impact of dioxin on humans, several mammalian models are considered but the actual impact of the chemical varies from species to species. In animals, exposure to dioxin leads to birth defects, but in humans the finding is not confirmed. Breastfeeding infants are more highly exposed than adults (US-EPA, 1994). The ingestion of the chemical pollutant is much more in a one year old infant than adults, but the total amount of dioxin ingested by the infant is much lower as compared to the average ingestion of dioxin by an adult (EPA, 2000; ATSDR, 1998).

In the year 1998, the WHO was consulted for the re-evaluation of the Total Dietary Intake (TDI) based on the Lowest Observed Adverse Effect Levels for the sensitive responses that can have adverse effects on the laboratory animals (van Leeuwen *et al*., 2000; WHO, 2000). The effects used to decide upon the TDI were immune suppression in offspring, decrease in sperm count, and genital malformation. High dioxin levels may cause darkening of skin, patches on the skin and skin lesions, and liver function impairment. Long-term exposure can lead to immune function impairment along with the failure of the nervous system, reproductive system, cardiovascular disease, diabetes, endometriosis, reduced testosterone, alteration in thyroid function, abnormalities in nail and tooth, metabolism related problems, and disruption in the endocrine system. Chronic exposure to the pollutant leads to cancer (Cole *et al*., 2003; Crump *et al*., 2003). These findings are consistent with experiments in lab animals.

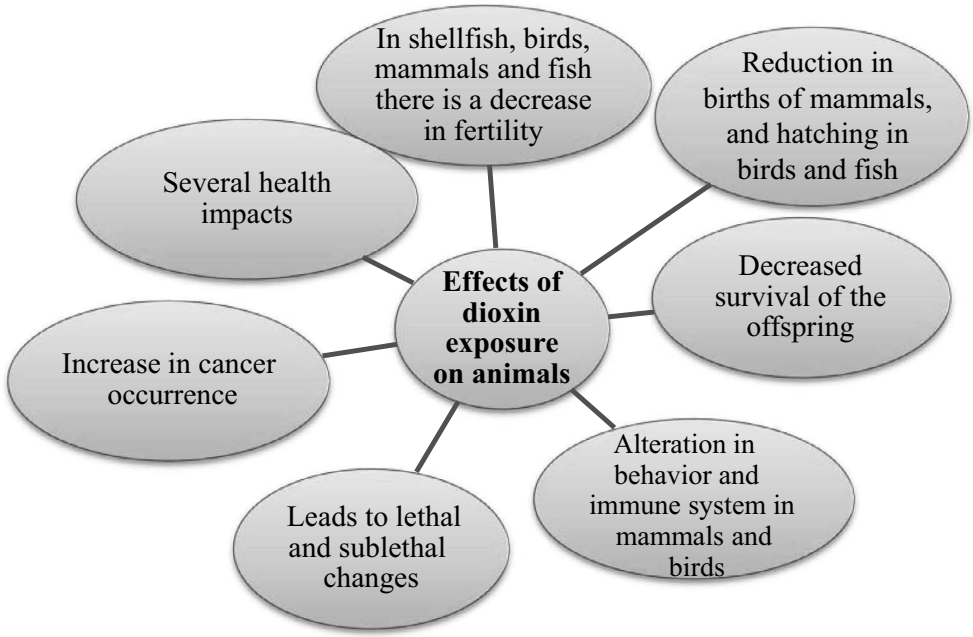


*Figure 9.2* Effects of dioxin exposure on animals.
(*Sources*: EPA, 1997; WHO, 2010; Raloff, 1997; Hornung *et al*., 1996)

*Table 9.1* Effect of dioxin on animals.

| *Pathological changes* | *Animal model* | *Reference* |
|---|---|---|
| Pathologic changes like liver damage (Icterus), hemorrhage, depletion of lymphoid organs. Degenerative changes moderate in kidney and thyroid glands, atrophy of adrenal, hyperplasia of urinary bladder, epithelium | Rats, guinea pigs | Gupta *et al*., 1973 |
| Chloracne | Human | Suskind (1985). |
| Endometriosis | Rhesus monkey | Rier *et al*.,1993 |
| **Cancer** | **Human** | Kogevinas, 2001 |

### 1.4 *Impacts on human health*

Many studies examine the effects of dioxin on the ecosystem, animals, and humans. Exposure to dioxin is linked to damage to the immune system, cardiovascular system, nervous system, liver, lungs, and reproductive system (APHA, 1996; Travis and Hattemer-Frey, 1991). Dioxin is linked to occurrence of cancer. According to the Indian Agency for Research on Cancer (IARC) 2,3,7,8-TCDD as a Group I carcinogen (IARC (IRAC, 1997 Anderson and Knapp., 2005).

The Institute of Medicine (2006) links TCDD to the occurrence of non-Hodgkin's lymphoma, chloracne, chronic lymphatic leukaemia, and soft tissue sarcoma. The progeny of

*Table 9.2* Reproductive effect in response to dioxin exposure (Adapted from Birnbaum and Toovisto, 2000).

| *Effect* | *Animal model* | *References* |
|---|---|---|
| Decreases in epididymal sperm numbers | Mouse | Theobald *et al*., 1997 |
| Reproductive disease endometriosis | Rhesus monkeys | Rier *et al*., 2013 |
| Daily sperm production permanently decreased | Rat | Faqi *et al*., 1998 |
| **Reduced Spermatogenesis** | Rat | Mably *et al*., 1992 |
| Impairs female reproduction via reduced steroid hormone secretion | Fish | King-Heiden *et al*., 2012 |
| Estrous cycle suppression and ovulation | Rat | Li, X.L., Johnson, D.C. and Rozman, K.K. (1995). |
| Malformations of the external genitalia | Rat | Gray J *et al*., 1997 |
| **Sexual Differentiation and sex ratio** | *Gobiocypris rarus* | Wu *et al*., 2011 |
| Fetal loss R(spontaneous abortions) | Rhesus monkey | Birnbaum and Toovisto, 2000 |

dioxin-exposed people are prone to prostate cancer, spinal bifida, lung cancer, cancer of the larynx, trachea, bronchus, prostate, amyloidosis, early onset of peripheral neuropathy, hypertension, Type-II diabetes mellitus, and porphyria cutanea tarda (Belazzi and Pexa, 2001).

2,3,7,8-TCDD is characterized as a potent "carcinogen" (U.S. EPA, 2000). Chlorinated dibenzo-p dioxins (CDDs), dioxins like polychlorinated biphenyls (PCFs), and chlorinated dibenzofurans (CDFs) are characterized as likely carcinogens (ATSDR, 1998; IARC, 1997). In humans, dioxin is carcinogenic; all types of cancers are prevalent in humans without any specific cancer dominating. The risk of cancer, such as breast cancer and testicular cancer, has been found to be dominant in the specific cohort, but there is an irregular pattern observed otherwise. The most common effects in humans include the risk of reproductive diseases and endocrine disruption (Kogevinas, 2001).

Chemical coordination and communication are a function of the endocrine system (ATSDR, 1998). Dioxins are endocrine disruptors, as they mimic hormones and lead to the alteration of the functions of hormones. The endocrine system helps in maintaining coordination throughout the body and aids in cell-to-cell communication (ATSDR, 1998). Endocrine disruption means that these hormones can change the regulatory functions of the endocrine system, immune system, and nervous system (U.S. EPA, 1997) Endocrine disruption leads to endometriosis and breast cancer in women, whereas in men it may lead to cancers of the prostate and testis, along with reduction in the sperm count, i.e. reduction in the fertility of males. Endocrine disruption also leads to changes in pituitary and thyroid gland function, suppression of the immune system, and affects neurological behaviour (U.S. EPA, 1997).

## 2 Common molecular mechanism of action

Dioxin exerts its mechanism of action in vertebrates by activating the aryl hydrocarbon receptor (AHR), which is a helix-loop-helix transcription factor and belongs to the PER-ARNT-SIM (PAS) superfamily of TFs (Gu *et al*., 2000). AHR belongs to the family of highly conserved proteins across the vertebrate species and also closely related proteins in invertebrates (Hahn., 2002). The AHR has an important role in the development of hypoxia, aging, and circadian rhythms (Carlson and Perdew, 2002). In the free state,

AHR exists in a cytoplasm bound with two molecules of chaperones, including heat shock proteins Hsp 90 and one molecule of prostaglandin E synthase (p23) and hepatitis B, which is like immunophilin XAP2 (Petrulis and Perdew, 2002).

TCDD enters the cell with the help of diffusion, binds to AHR, along with one molecule of Hsp 90, and this leads to the dissociation of both p23 and XAP 2 from AHR. The AHR translocates in the nucleus and hetrodimerizes with the AHR nuclear translocator (ARNT) along with the PAS domains, which leads to the release of the Hsp 90 molecule before the transcription takes place. AHR, TCDD, and ARNT form a complex and bind to DNA at the dioxin response element (DRE) (Beischlag *et al*., 2005). AHR leads in the control of nucleur steroid receptors and control of the cell cycle (Puga *et al*., 2009).

The biochemical changes which occur include the alteration in expression of enzymes involved in the metabolism and the growth factors and their receptors (Lambert *et al*., 2006; Mc Hale *et al*., 2007). At the time of development, there is alteration in the thyroid and immune function and behavioral changes, which include hearing, delay of breast development, and psychomotor function, brain, and neural development delays, which lead to problems in cognition, dental problems, and development of reproductive organs and sex ratio alteration. The thyroid stimulating hormone was found to be increased in the mothers of the neonates who have elevated levels of dioxin in the plasma (Baccarelli *et al*., 2008). In males exposed to the pollutant, there is a reduction in the sperm counts and motility during puberty (Mocarelli *et al*., 2009).

### 2.1 *Control of dioxin pollution*

Dioxin pollution has received attention worldwide. According to the United Nation's Environmental Program 12 persistent organic pollutants (POPs) like dioxin have been identified and a global treaty has been finalized to minimize the effects of pollution and to ban the pollutants. Food, especially meat and fish, has been contaminated with dioxin. The EPA has been working on this issue and has been successful in a 12-year study on dioxin, which named dioxin as a much more potentially hazardous pollutant than previously thought, and it has been listed as targeted PBTs by the EPA. Current projects of Ecology's Hazardous waste and Toxics Reduction Program to reduce the dioxin level or to nullify their level works on Ecology's Hazardous Waste and Toxics Reduction Program has been working in many projects to reduce and eliminate dioxins. These strategies might reduce the load of dioxin pollution in the environment**. The Hazardous Waste and Toxics Reduction Program has the following agenda to reduce the load of dioxin-like contaminants**:

- Standard should be set for using fertilizers and steel mill flue dust used in them.
- Cement kill dust management.
- Micronutrient fertilizers and waste derived fertilizers should be screened for dioxin.
- Management of wood-fired boiler ash.
- Elimination and reduction of pollutants present in the environment.

## 3 Conclusion

The toxin dioxin and its related chemical compounds are the outcome of industrial pollution, which is ubiquitous as well as persistent in the environment. The principle

environmental sources of dioxin and dioxin-like compounds are emissions from industries, incineration, combustion, and several industrial processes like paper mills, grass fires, fireplaces, etc. Dioxins are fat soluble and accumulate in the body of animals as well as humans. Extensive use and misuse of dioxins have created havoc in the environment. The persistence of pollutants contributes to their build up in the environment. Animals accumulate dioxin primarily through ingestion of contaminated vegetation and soil, further it passes to the next level of the food chain. Dioxin in animals is, in addition to cancer, linked to several adverse human health effects such as immunologic, reproductive endocrine toxicity, and developmental issues. The aerial transport of these emissions is the primary pathway by which dioxins enter the terrestrial environment and food chain. Impacts on biota and the environment have made it mandatory for imposing stringent laws, which limits the use of the pollutant and such pollutants shall be treated as toxic and hazardous waste and should be destroyed.

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# Remediation of dioxin

## Past, present, and future

Chapter 10

# Dioxin, a serious environmental threat

## Methods of removal

*Gomaa A.M. Ali, Milad Chahardori and Hamidreza Sadegh*

## 1 Introduction

Dioxins are organic compounds that are mostly derived from inadvertent industrial processes, while a small amount of them is of natural origin. These compounds are known as toxic due to their structures, which consist of two benzene rings connected by polychlorinated dibenzofurans or two polychlorinated dibenzo-*p*-dioxins or two benzene rings bonded together by a single carbon-carbon bond, with chlorine on the different positions (Figure 10.1) (Safe, 1990; de Lacerda, 2019). Dioxins are resistent to physical-chemical and biological degradation, low volatility, the ability to be transported to isolated parts of the planet, and propensity of bioaccumulation in the fat tissue of living organisms (Poiger and Schlatter, 1979; Assefa *et al*., 2019). Once dioxins are transferred to the environment (e.g. sediment of oceans, rivers, and to the topsoil or even plant leaves) they can be exposed to humans and animals in the gas phase or through food chains.

The World Health Organization (WHO) estimates that 98% of human exposure to dioxin-like compounds is via food consumption (Van den Berg *et al*., 2006; WHO, 2010). Health disorders that have been related to dioxin exposure include chloracne, cardiovascular diseases, thyroid dysfunctions, change in the sex ratio of offspring, and even cancer. The most specific sign of abnormal dioxin exposure is the skin lesions (chloracne) which is classically used as a key marker in humans (Saurat *et al.*, 2011). Since the early 1990s, WHO has organized expert meetings to address the toxic equivalency factors (TEFs) for dioxin and dioxin-like compounds on the international level, thereby giving

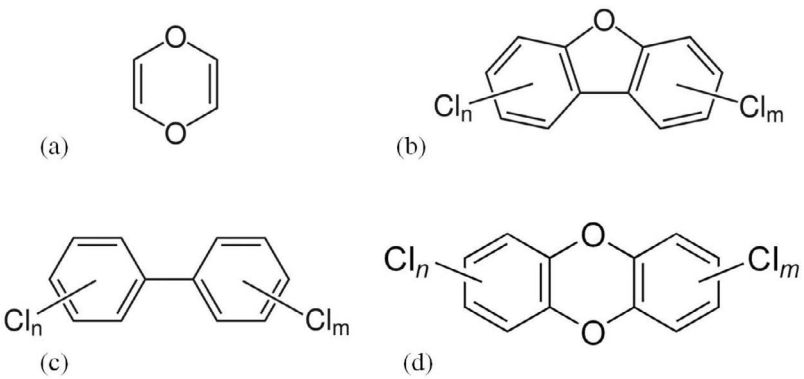


*Figure 10.1* Dioxins compounds; (a) 1,4-dioxin, (b) polychlorinated dibenzofurans, (c) polychlorinated biphenyls and polychlorinated dibenzo-p-dioxins (d).

recommendations to national regulatory authorities. In 2005, WHO initially reported a global burden of foodborne disease. Dioxin's effects on fertility and thyroid functionality were discussed in this context. Also, the European Union (EU) Commission published the first legal document (No 252/2012) regarding set extensive validation criteria for screening dioxins and dioxin-like compounds in food via cell-based bioassays to achieve minimum standards throughout the laboratories. These studies provided a better understanding of the food groups that should receive more attention from the authorities and to dietary exposure temporal trend development.

## 2 Source of dioxins

One of the largest sources of dioxin production and emission in the environment is the process of burning municipal waste. Around 98% of human exposure to dioxin compounds comes via food consumption (WHO, 2010). Researchers have described two general mechanisms for the formation of dioxins: (i) from chemically similar precursors (chlorobenzenes and chlorophenols) and (ii) the so-called de novo synthesis (Altwicker, 1996). Hospital waste includes bands, blood tubes, etc., from the sources of these compounds (Stanmore and Clunies-Ross, 2000). Cement kilns, wood burning, vehicles, and coal can be major sources of dioxin production. According to reports about dioxins, burning wood is about 945 g I-TEQ/year, which is more than coal, but because coal is used more it has a greater effect on the production of these compounds (Harrad *et al*., 1991; Chen, 2004; Lavric *et al*., 2004; Eduljee, 1999; Abad *et al*., 2004). It is worth mentioning that the production of chemicals is another source of emissions, including polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), which can be called lateral products of chlorine compounds, such as phenol-chlorine, phenoxy herbicides (Öberg *et al*., 1992; Sidhu and Edwards, 2002). High levels of PCDDs and PCDFs were detected in free-range eggs (Piskorska-Pliszczynska *et al*., 2016). The hydrophobic nature of these compounds make it possible to locate these compounds at the disposal site at high waste disposal sites (Rotard *et al*., 1994; Kjeller and Rappe, 1995).

## 3 Toxicity of dioxins

In toxicity studies, it has been shown that because of their chronic implications on biota, PCDDs and PCDFs are highly toxic and carcinogenic to an ecosystem (Rathna *et al*., 2018). By examining diseases that are exposed to the composition of dioxins, especially tetrachlorodibenzofuran (TCDD), they found that humans with an average body fat content of about 24% should be some of the most resistant of LD50 of $\approx$12.5 mg/kg. However, by studying patients, scientists found that LD50 of the human body for TCDD may be significantly less than predicted. The toxic effects of TCDD are due to their high affinity and long elimination half-lifes (Sorg *et al.*, 2009; Geyer *et al.*, 1993). Where, they accumulate in the lipids in tissues on a physical basis by simple partitioning.

## 4 Removal methods of dioxin compounds

### 4.1 *Using sulfur compounds*

One of the effective ways to remove dioxins is using sulfur compounds such as $[(NH_4)_2SO_4]$, $FeS_2$ and many different sulfur compounds are good detergents, as well as $SO_2$ as an

inhibitor. These inorganic sulphur compounds can be used as an active suppressant in awaste incineration facility. In addition, the mechanism involves removal of the cholorine from polychlorinated dioxins in to HCl ($SO_2$+$Cl_2$+$H_2O$→$SO_3$+2HCl) is the main mechanism of the inhibition of dioxin (Mukherjee *et al.*, 2016). Moreover, it has been stated that the sulfur content in fuel can be used to reduce dioxin formation, which depend on the sulfur to cholorine ratio (Anthony *et al.*, 2001).

### 4.2 *Adsorption on active charcoal*

The polychlorinated dibenzo-p-dioxins and dibenzofurans are a group of persistent organic compounds that are highly toxic and carcinogenic compounds that are produced by the burning of industrial waste that is used to remove these gases from active charcoal. The amount of active coronal injection is related. For example, 20, 40 and 50 kg/h active carbon eliminated these compounds at 86, 96 and 97%, respectively. For the minimum amount of activated carbon used, different concentrations, temperatures, and times need to be considered (Mukherjee *et al*., 2016). Kawashima *et al*. examined the amount of dioxin adsorption by dispersing carbon, and their results showed that the active carbon has a magnitude of 700–1200, and micropores with a diameter of approximately 0.7–0.8 have the highest absorption (Guo *et al*., 2016). In a similar study, the combination of countercurrent supercritical $CO_2$ extraction and activated carbon treatment optimized the removal of PCDD from fish oil Guo et al. found that the presence of the functional groups on benzene compounds increases the absorption of gases, such as methyl and chlorine increase the amount of nitrogen, and vice versa, the phenol node reduces the amount of carbon uptake by oxidation (Kawashima *et al.*, 2009). Eighty percent of the dioxin-like PCB could be removed from fish oil by activated carbon (Maes *et al.*, 2005).

### 4.3 *Supercritical CO2 and activated carbon*

It is well known that omega-3s, such as eicosapentaenoic acid and docosahexaenoic acid, are very important in human health. Since fish oil is the main source of these compounds, they have been contaminated with persistent organic pollutants, especially polychlorinated dibenzo-p-dioxins and dioxin-like polychlorinated biphenyls. Supercritical $CO_2$ and activated carbon were used to remove these compounds, such as the polychlorinated dibenzo-p-dioxins and dioxin-like polychlorinated biphenyls, from fish oil. The absorbent supercritical $CO_2$ was at a temperature of 70° and a pressure of 30 MPa and, with a $CO_2$/oil ratio, eliminated 93% of polychlorinated dibenzo-p-dioxins and 85% of dioxin-like polychlorinated biphenyls, and eliminated 94 and 93% of dioxin-like polychlorinated biphenyls, respectively. The results of this study showed that the active carbon was better (Kawashima *et al*., 2009). The supercritical $CO_2$ extraction and activated carbon treatment system components are shown in Figure 10.2. The combination of countercurrent supercritical $CO_2$ extraction and activated carbon treatment is applicable to the removal of PCDDs, PCDFs, and DL-PCBs from fish oil.

### 4.4 *Facilitated supported ionic liquid membranes*

Ceramic membranes containing specific ionic liquids were used for dioxin removal from incineration sources (Kulkarni *et al*., 2012). This process showed a good stability at very high temperatures. The membranes were prepared by successfully immobilizing the ionic liquids inside the porous structure of ceramic membranes. The obtained results

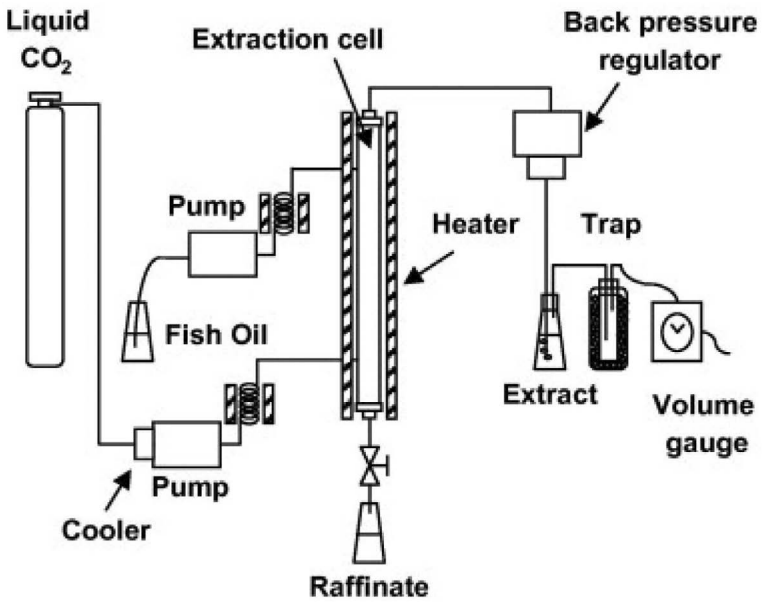


*Figure 10.2* Schematic diagram of supercritical $CO_2$ extraction and activated carbon treatment for dioxins and dioxin-like PCBs removal from fish oil.

(Source: Copied with permission from Ref. (Kawashima *et al*., 2009)

recommended the potential of using ceramic membranes with immobilized ionic liquids for the removal of dioxins from high-temperature vapor sources.

### 4.5 *Microbial degradation*

Aerobic bacteria such as Sphingomonas, Pseudomonas, and Burkholderia were used to degrade lower chlorinated dioxins (Field and Sierra-Alvarez, 2008). Unique angular dioxygenases are used as degradation initiators. Chlorinated dioxins can also be attacked cometabolically under aerobic conditions by white-rot fungi that utilize extracellular lignin degrading peroxidases. Complete removal of dibenzofuran and 81% of dibenzo-p-dioxin was obtained when *Sphingomonas wittichii* strain was used (Nam *et al*., 2005).

### 4.6 *Ethanol washing*

Another method to remove dioxin compounds is by washing with ethanol. Jonsson *et al*. used flushing with ethanol to remove PCDD and PCDF compounds from soils. Several factors influenced removal efficiency, such as temperature, time, and ethanol concentration. Screening experiments showed that ethanol concentration is the most important parameter. Four different soil types were tested, each of these soils having the same washing conditions (10 times with 75% alcohol at 60 °C). Removal efficiencies from 81% and 85% were obtained. For soils rich in organic compounds, removal efficiencies were up to

97%. The removal of these materials depends on the concentration distribution of the particles (Jonsson *et al*., 2010).

### 4.7 *Washing and ball-milling treatment method*

Li *et al*. used a washing and ball-milling treatment method for the removal of dioxins in ash. This method was used to remove chlorine and reduce the amount of dioxin. The results showed that more than 90% of particles were in the range of 1 to 50 mm, and most of the dioxin compounds in the range of 0.030 and 0.070. Na, Ca, K, and Br would be removed in the washings. However, dioxin and other solid metals remained. Washing and Fe/Ni-$SiO_2$ ball-milling removed up to 93.20% of dioxin. This seemed to be the most effective and the best choice to remove dioxins (Li *et al*., 2017).

### 4.8 *Thermal plasma method*

The thermal plasma method is another method for eliminating toxic dioxin compounds. A pulsed positive discharge was carried out on ash containing dioxins, and the results of the study found that compounds with different isomers and higher levels of toxicity were further degraded. The degradation efficiency varied from 80% to 20%. Among all the isomers in the ash, there was 2,3,7,8-T4CDD with the highest toxicity, and the highest degradation efficiency of 81% for removal has also been shown. Also, with the concentration of toxic compounds, the efficiency of elimination increased (Zhou *et al*., 2003).

### 4.9 *Ultraviolet mercury lamps*

Several studies have been done to eliminate dioxins in the environment, one of which has been carried out by Kostantinov *et al*. using ultraviolet mercury lamps. Experiments with 1,6-[3H]-2,3,7,8-TCDD revealed that the principal photolytic pathway involves cleavage of CeO bonds rather than CeCl bonds, giving chlorinated hydroxydiphenyl ethers as the initial products. The photolysis products from 2,3,7,8-TCDD do not bind to either the rat hepatic Ah receptor or the estrogen receptor in vitro, making it unlikely that the products from the UV treatment of PCDD/PCDF in laboratory waste will show either Ah or estrogen receptor-mediated toxicological effects (Konstantinov *et al*., 2000).

## 5 Conclusion

Dioxin compounds are highly hazardous to both humans and animals due to their high environmental toxicity. The resulting effects contribute to the sensitivity and safety of the body. In recent years, many sources produce these compounds, such as the production of cold drops, chlorine released from paper bleaching, and burning of urban solids. To remove these materials, various types of adhesives have been proposed, including using sulfur compounds, activated charcoal, thermal plasma method, ethanol washing, ball-milling treatment method, and microbial degradation. Removing these compounds from the environment is crucial to reduce its harmful impact on humans and animals. In addition, safe and clean food via good controls and practices during primary production, processing, distribution, and sale is essential

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Chapter 11

# A review of health hazards and remediation techniques of dioxins

*Sadaf Fatima and Md. Osaid Alam*

## 1 Introduction

Polychlorinated dibenzo p-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs) are noxious, teratogenic, and carcinogenic chemicals that occur in minute quantity in the environment. PCDD and PCDF are binaries of chlorinated polycyclic aromatic hydrocarbons (CPAHs), having similar molecular structure and physicochemical properties (Fries, 1995). CPAHs are double benzene rings linked by a mono-oxygenated ring for PCDF or a di-oxygenated ring for PCDD. The chlorine atoms may hold one to eight distinct sites on the benzenic rings in PCDD and PCDF compounds (INSERM, 2000). There are 210 congeners of dioxins based on the number and positions of chlorine atoms, out of which there are 135 distinct PCDF congeners and 75 PCDD congeners (CCME, 2002). In 17 out of 210 congeners, chlorine atoms occupy 2, 3, 7, and 8 loci of the mother molecules and are very toxic to human beings. For instance, 2,3,7,8-TCDD (2,3,7,8-$Cl_4$DD) is the utmost toxic synthetic dioxin compound (Kumar *et al*., 2013; Gillbreath and McKee, 2015). Dioxins and furans have been classified into three main categories: polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs) (Figure 11.1). They emerge as by-products in organochlorides production, in the bleaching of paper, during the incineration of chlorine-bearing materials, for instance, polyvinyl chloride (PVC), as well as from natural origins such as wildfires (Wikoff and Urban, 2013; Kanan and Samara, 2018).

The US Environmental protection agency (USEPA) has recognized dioxins as extremely carcinogenic because they reduce our immunity and cause hormonal imbalance. Traces of dioxins have been detected in the tissues of the local population living nearby to industrial areas (Gasiewicz and Park, 2003). Therefore, dioxins must be removed from flue gases before their discharge into the environment, and soil/sediments/wastes should also be

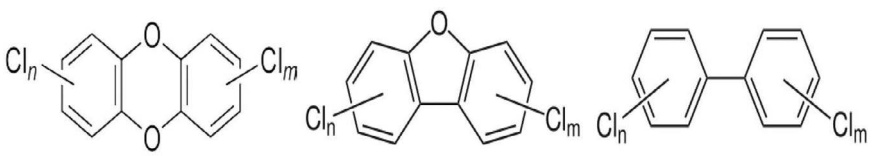


*Figure 11.1* Chemical structure of PCDD, PCDF, and PCB.

*Table 11.1* Dioxins emission from common combustion sources.

| *Combustion Sources* | *Dioxins in Flue Gas (ng-TE/Nm$^3$)** | *References* |
|---|---|---|
| Gas combustion | 0.07–100 | Dudhuis *et al.* (1990), Christmann *et al.* (1989) |
| Municipal waste incineration | 0.2–63 | Johnke and Stelzner (1992) |
| Natural wood | 2.7–14 | Vikelsoe *et al.* (1994), Dumler-Gradl *et al.* (1993) |
| Wood combustion | 0.02–1.8 | Schatowitz *et al.* (1994), Kolenda *et al.* (1994) |
| Hazardous waste incineration | 0.1–0.5 | Schoner (1992) |
| Oil combustion | 0.03–0.3 | Taucher *et al.* (1992) |
| Oil furnace | 3.5 (pg/L-oil) | Marklund *et al.* (1990) |
| Plastic pyrolysis | Detected | De Fre and Rymen (1989) |

**TE refers to I-TEQ (international toxic equivalent quantity)*

treated after contamination due to their highly toxic nature and potential to build up in the living tissues (Stanmore, 2004; Kaivosoja *et al*., 2012).

## 2 Sources of dioxins

The prominent dioxins sources in the environment can be divided into four major categories (Kulkarni *et al*., 2008; Kanan and Samara, 2018): (a) combustion sources (wood burning, diesel vehicles, cement kilns, crematoria utilities, coal-fired utilities); (b) incineration sources (bio-medical waste incinerators, hazardous waste incinerators, municipal waste incinerators, sewage sludge); (c) industrial sources (chemical manufacturing units, pulp and paper mills, metal industry, power boilers); and (d) reservoir sources (biochemical and photolytic processes, landfill burning, wildfires, accidental release).

Dioxins have been mostly released from combustion sources. Municipal waste combustion, waste incineration, residential wood combustion, burning salt-laden wood, coastal pulp mill boilers, iron sintering, and electrical arc furnace steel manufacturing together are found to generate 80% of the total dioxins emissions (Loth *et al*., 2005). Table 11.1 presents an overview of the dioxin emissions in the flue gas released from various combustion sources (Huang and Buekens, 1995).

### 2.1 *Formation of dioxins*

#### 2.1.1 Precursors of dioxins formation

Chlorinated compounds such as chlorobenzenes, chlorophenols, polycyclic aromatic hydrocarbons, and de novo reactions of fly ash are the precursors of dioxins (Addink *et al*., 1998; Hatanaka *et al*., 2004; Tuppurainen *et al*., 2003; Fujimori *et al*., 2010). The formation of precursor compounds takes place in the post-combustion zone (Froese and Hutzinger, 1996). Soot or fly ash, and residual carbon are among the leading sources of PCDD/Fs formation (Addink and Olie, 1995) as PCDD/Fs emission is directly proportional to the rate of carbon consumption (Kuzuhara *et al*., 2003). The factors affecting PCDD/Fs formation

include residence time, combustion temperature, precursors in feed, chlorine in feed, PCDD in feed, processing of feed, supplemental fuel, and availability of oxygen. (Mckay, 2002). Precursor formation of PCDD/Fs and de-novo synthesis of PCDD/Fs are presented in Table 11.2 and Table 11.3, respectively (Huang and Buekens, 1995; Mukherjee *et al*., 2016).

*Table 11.2* Precursors formation of PCDD/Fs.

| *Gas-phase material* | *Solid-phase material* | *Temperature (K)* | *Solid-phase residence time (min)* | *References* |
|---|---|---|---|---|
| Chlorophenols moreover, blend of nitrogen and oxygen | CuO | 673 | 30 | Gullett *et al.* (1992) Huang and Buekens (1995), Mukherjee *et al.* (2016) |
| 2,3,4,6-$T_4$CP, nitrogen, and oxygen | Fly ash | 598 | 2–15 | Altwicker and Milligan (1993), Huang and Buekens (1995), Mukherjee *et al.* (2016) |
| C-$P_6$CP and air | – | 573 | 60 | Naikwadi *et al.* (1993) Huang and Buekens (1995), Mukherjee *et al.* (2016) |
| 2,4,6-$T_3$CP and water | Active carbon | 573 | 30 | Luijk *et al.* (1994) Huang and Buekens (1995), Mukherjee *et al.* (2016) |
| C-$P^5$CP and air | Blend of charcoal, silica gel, and $CuCl_2$ | 573 | 10 to 60 | Dickson *et al.* (1992) Huang and Buekens (1995), Mukherjee *et al.* (2016) |

*Table 11.3* De-novo synthesis of PCDD/Fs.

| *Gas-phase material* | *Solid-phase material* | *Temperature* | *Solid-phase residence time (min)* | *Author* |
|---|---|---|---|---|
| Air, water, and hydrogen chloride | Active carbon and $CuCl_2$ | 573 | 60 | Luijk *et al.* (1994) Huang and Buekens (1995), Mukherjee *et al.* (2016) |
| Air | Fly ash | – | 120 | Naikwadi *et al.* (1993) Huang and Buekens (1995) Mukherjee *et al.* (2016) |
| Oxygen (10%) and nitrogen | Fly ash | – | 30 | Milligan and Altwicker (1993), Huang and Buekens (1995) |
| C-$P_5$CP and air | Mix of charcoal, silica gel, and $CuCl_2$ | – | 10 to 60 | Dickson *et al.* (1992) Huang and Buekens (1995), Mukherjee *et al.* (2016) |

### 2.2 *Mechanism of formation*

There are two main mechanisms of dioxin formation (PCDD/Fs). The first mechanism involves homogeneous reactions, i.e. rearrangement reaction of precursor compounds like chlorobenzenes (CBs) and chlorophenols (CP) at temperatures of 500 °C −800 °C in the gas phase. The PCDD/Fs formed under this mechanism are termed high-temperature PCDD/Fs or homogeneous PCDD/Fs. The second reaction involves heterogeneous reactions and the mechanism includes the surface catalytic effect of soot or fly ash, which is known as a de novo process that occurs at temperatures 200 °C −400 °C in the post-combustion zone. PCDD/Fs thus formed are called as low-temperature PCDD/Fs or heterogeneous PCDD/Fs (Huang and Buekens, 1995; Addink and Olie, 1995; Stanmore, 2004; Liu *et al*., 2013; Vermeulen *et al*., 2014; Mukherjee *et al*., 2016).

## 3 Health hazards of dioxins, furans, and polychlorinated benzene

Dioxins and alike compounds are not soluble in water but highly oil-soluble, thus extremely persistent and bioaccumulative in fatty tissues and are biomagnified in concentration as they move from lower trophic levels to higher trophic levels. Due to volatilization and cold condensation properties, dioxins may also be carried to the glacial or polar regions of the earth by global atmospheric circulation (Carey *et al*., 1998). A high level of body fat is found in animals of cold regions to insulate their bodies from the very low temperatures. However, they bioaccumulate a large number of organic toxins, including dioxins, in this fat. The half-life of dioxin elimination varies from as little as six months for some PCDFs to as much as 20 years for others (Schecter *et al*., 1990; Flesch-Janys *et al*., 1995; Ryan *et al*., 1993; Ogura, 2004). In the human body, the half-life for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) varies between 7–12 years with wide variation from person to person (ATSDR, 1998). Recently, pharmacokinetic investigations have revealed that the dioxin's half-life is dose-dependent, i.e. dioxin's elimination increases with increasing concentration. It also depends upon the body anatomy, so that an elevated level of body fat leads to enhanced dioxin persistence (Aylward *et al*., 2005; Emond *et al*., 2005). To establish the relative toxicities, WHO (2005) assigned dioxin toxic equivalency factors (TEFs) to PCDDs, PCDFs, and some PCBs based on their relative toxicities compared to the most lethal dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is assigned a TEF value of 1 and used as a reference (Van den Berg *et al*., 2006) (Table 11.4). The effects of dioxins are additives due to the similar mechanism of action for these compounds. Thus, the TEFs for each compound can be summed to estimate the TEF for the conglomeration of dioxins. TCDD, the most toxic dioxin, is well known for its application in the Agent Orange herbicide used in the historic Vietnam War (IOM, 2005). There are some of the meagrely toxic dioxins such as PCBs with TEF value of 0.0001, yet, they may still be of concern due to their much larger amounts than other dioxins of higher TEFs.

In vertebrates including humans, dioxins and similar chemicals have been known to cause a number of chronic effects such as lack of immunity (Weisglas-Kuperus *et al*., 2000); reproductive and developmental oddities (Guo *et al*., 2003); cancer (Fingerhut *et al*., 1991; Steenland *et al*., 1999); thyroid disorders (Pavuk *et al*., 2003); endocrine disruption, including diabetes (Longnecker and Michalek, 2000), central and peripheral nervous system pathology (Guo *et al*., 2003); bronchitis and reduced pulmonary functions

*Table 11.4* WHO (2005) toxic equivalency factor (TEF) of dioxins (Van den Berg *et al*., 2006).

| | *Chemical compound* | *TEF* |
|---|---|---|
| Chlorinated Dibenzo-p-dioxins | OCDD | 0.0003 |
| | 1,2,3,4,6,7,8-Hepta-CDD | 0.01 |
| | 1,2,3,4,7,8-Hexa-CDD | 0.1 |
| | 1,2,3,6,7,8-Hexa-CDD | |
| | 1,2,3,7,8,9-Hexa-CDD | |
| | 1,2,3,7,8-Penta-CDD | 1 |
| | 2,3,7,8-Tetra-CDD | |
| Chlorinated Dibenzofurans | OCDF | 0.0003 |
| | 1,2,3,4,6,7,8-Hepta-CDF | 0.01 |
| | 1,2,3,4,7,8,9-Hepta-CDF | |
| | 1,2,3,7,8-Penta-CDF | 0.03 |
| | 1,2,3,4,7,8-Hexa-CDF | 0.1 |
| | 1,2,3,6,7,8-Hexa-CDF | |
| | 1,2,3,7,8,9-Hexa-CDF | |
| | 2,3,4,6,7,8-Hexa-CDF | |
| | 2,3,7,8-Tetra-CDF | |
| | 2,3,4,7,8-Penta-CDF | 0.3 |
| Mono-ortho-PCBs | 2,3',4,4',5,5'-HxCB | 0.00003 |
| | 2,3,3',4,4',5,5'-HpCB | |
| | 2',3,4,4',5-PeCB | |
| | 2,3',4,4',5-PeCB | |
| | 2,3,3',4,4'-PeCB | |
| | 2,3,3',4,4',5'-HxCB | |
| | 2,3,3',4,4',5-HxCB | |
| | 2,3,4,4',5-PeCB | |
| Coplanar PCBs | 3,3',4,4'-TCB | 0.0001 |
| | 3,4,4',5-TCB | 0.0003 |
| | 3,3',4,4',5,5'-HxCB | 0.03 |
| | 3,3',4,4',5-PeCB | 0.1 |

(Nakanishi *et al*., 1985; Shigematsu *et al*., 1978); change in serum testosterone level (Egeland *et al*., 1994); eyelid pathology, including meibomian gland hypersecretion and hyper pigmented conjunctivae; loss of appetite; vomiting; nausea; skin rashes, chloracne or acne; hypertrichosis; liver damage; elevated serum cholesterol and triglycerides (Kimbrough *et al*., 1977) gum pigmentation (Masuda, 2003); and enamel hypo mineralization of permanent first molars in kids (Alaluusua *et al*., 2004). Moreover, elevated mortality risk was correlated with excessive industrial exposure to dioxins with acute ischemic cardiovascular incidents (Flesch-Janys *et al*., 1995). Kimbrough and Jensen (1989) reported transient acute health effects viz. irritability, fatigue, pruritus, headache, and pain in the abdomen, inability to have erections or ejaculations, personality changes, insomnia, and diarrhea especially following industrial exposures. Clinical manifestations and associated dioxins have been summarized in Table 11.5.

### 3.1 *Human cancer*

Based on findings of several studies on animals as well as humans, the EPA (2000) characterized dioxins and alike chemical compounds, i.e. chlorinated dibenzo-p-dioxins

*Table 11.5* Clinical manifestations associated with dioxin and furan compounds.

| *Clinical manifestation* | *Chemicals* | *References* |
|---|---|---|
| Cancer | 2,3,7,8-TCDD, PCBs | Fingerhut *et al.* (1991), Steenland *et al.* (1999), Chen *et al.* (1992) |
| Thyroid | 2,3,7,8-TCDD | Pavuk *et al.* (2003) |
| Diabetes | 2,3,7,8-TCDD | Longnecker and Michalek (2000) |
| Elevated serum cholesterol and triglycerides | 2,3,7,8-TCDD | Kimbrough *et al.* (1977) |
| Liver damage | | |
| Skin rashes | | |
| Hypertrichosis | | |
| Gum pigmentation | | |
| Eyelid pathology | | |
| Nausea | | |
| Loss of appetite | | |
| Vomiting | | |
| Change in serum testosterone | 2,3,7,8-TCDD | Egeland *et al.* (1994) |
| Enamel hypo mineralization of permanent first molars in children | 2,3,7,8-TCDD | Alaluusua *et al.* (2004) |
| Mortality from ischemic heart disease | 2,3,7,8-TCDD, PCDD/F | Flesch-Janys *et al.* (1995) |
| Mortality from cardiovascular disease | | |
| Cancer mortality | PCDD/F | |
| Immune deficiency | PCB congeners (118, 138, 153, 180), PCDD/F | Weisglas-Kuperus *et al.* (2000) |
| Developmental abnormalities | TCDD | Guo *et al.* (2003) |
| CNS and PNS pathology | PCBs, PCDFs | |
| Reproductive abnormalities | | |
| Pruritis | | |
| Fatigue/general malaise | | |
| Headache | | |
| Decreased pulmonary function and bronchitis | PCBs, PCDFs | Shigematsu *et al.* (1978), Nakanishi *et al.* (1985) |
| Hyper pigmented conjunctivae | PCBs | Masuda (2003) |
| Meibomian gland hypersecretion | | |

(CDDs), chlorinated dibenzofurans (CDFs), and polychlorinated biphenyls (PCBs) as "human carcinogen." In 1997, the International Agency for Research on Cancer (IARC, 1997) reported that 2,3,7,8-TCDD is a "known human carcinogen" (group 1). Later in 1998, the US Department of Health and Human Services also concluded that 2,3,7,8-TCDD "might cause cancer" (ATSDR, 1998). In some studies, risks of some specific kinds of cancer, i.e. multiple myeloma, lymphomas, soft tissue sarcoma, cancers of lungs, liver, testes, breast, and endometrium were reported being increased in dioxin exposed populations, although the results were not consistent between studies. Kogevinas *et al*. (1997) reported the elevated risks of breast cancer in men and women, endometrial cancer, and testicular cancer in men from exposure to dioxins. Fetuses, newborns, and children may be more vulnerable to dioxin exposure owing to speedy growth and development. The EPA (1994) established that newborns nursing for one year could consume more

dioxin than background exposure, i.e. what an adult typically ingests. Though, the overall dioxins the infant may consume during nursing would be lesser compared to the projected mean lifetime dose. Although breast milk seems to be a primary source of dioxin exposure to nursing infants, a large number of studies indicated the health benefits of breastfeeding even with the dioxin presence (ATSDR, 1998; EPA, 2000a, b).

### 3.2 *Endocrine, reproductive, and developmental effects*

The endocrine system is accountable for chemical communication and coordination all over the body (ATSDR, 1998). Some chemical compounds, such as dioxins, may disturb the endocrine system. These chemicals are termed as "endocrine disruptors" since they may mimic natural hormones, block hormonal action, or change the typical regulatory function of the immune, endocrine, and nervous systems (EPA, 1997). Endocrine disruption may result in prostate and testicular cancers in men, endometriosis and breast cancer in women, abnormal sexual development, reduced male fertility, immune suppression, disturbance in thyroid and pituitary gland functions, and neurobehavioral effects (EPA, 1997). Egeland *et al*. (1994) found decreased testosterone and increased gonadotrophin concentrations in workers exposed to high TCDD concentration.

### 3.3 *Minimizing the risk of exposure to dioxins*

There are several strategies which can help in minimizing the risk of exposure to dioxins. Some of these measures are as under:

1 A balanced diet that is low in saturated and total fats may help minimize any potential risk of dietary exposure to dioxins.
2 By replacing polyvinyl chloride (PVC) with alternative, chlorine-free materials, PVC-related dioxin pollution can be prevented.
3 All products made up of or packed in polyvinyl chloride (PVC) plastic should be avoided.
4 Chlorine containing weedicides and insecticides, especially chlorophenol weedicides, such as 2,4-D, should be avoided.
5 "Permethrin" flea sprays for pets should be avoided.
6 All chlorine-bearing organic chemicals, for example, the wood preservative pentachlorophenol, which is a widely used dioxin bearing household chemical, should be avoided.
7 Avoid bleached paper products and replace chlorine bleach (sodium hypochlorite) and goods containing it with oxygen bleach.
8 Unbleached (brown) coffee filters or those that have been bleached with non-chlorine bleach are recommended.
9 The use of Saran Wrap and analogous "cling-type" plastic wraps should be avoided unless they are surely a polyethylene.
10 Besides washing vegetables and fruits thoroughly to remove residual chlorophenol pesticide, it is recommended to use organic fruits and vegetables, i.e. grown without synthetic pesticides.
11 Since cotton is sprayed with chlorophenol insecticides, thus, the products containing cottonseed oil as a constituent (e.g., potato fries) should be deterred.

12 Avoid the tallow containing soaps, as it is a kind of animal fat.
13 "Triclosan" containing deodorants and soaps should be avoided because "triclosan" is a chlorophenol.
14 Smokers should roll their cigarettes in their unbleached paper.

## 4 Remediation techniques for dioxins removal

The dioxins and similar chemicals are eliminated from flue gas, sediments, wastes, and fly ash using several technologies.

### 4.1 *Removal of dioxin from flue gas*

In the current era, the quantities of generated waste have increased drastically, which is attributed to a rise in the living standard. Incineration technology has been one of the oldest methods to cut the wastes load, but emission of dioxin from flue gas is one of the major disadvantages of the method (McKay, 2002; Weber *et al*., 2002b). To overcome this, several processes and technologies are being used to control dioxin emissions in the flue gas – like a collection of particulate matter using fabric filter, scrubber and electrostatic precipitator (ESP), the addition of appropriate inhibitors, selective catalytic reduction, and good combustion practices braced with end-of-pipe treatment and other hybrid technologies. Dioxin concentrations in the flue gases ranged from 1 to 500 ng I-TEQ/$m^3$. Thus, the treatment of flue gas is critical for cutting down dioxin concentrations to an acceptable limit, i.e. 0.1 ng I-TEQ/$Nm^3$, before the discharge into the troposphere (Kulkarni *et al*., 2008). The technologies being used to eliminate dioxins from flue gas are discussed below:

#### 4.1.1 Particulate matter collection method

*Principle mechanism:* A variety of dust collectors are used during the incineration process to remove particle-bound dioxins (Kulkarni *et al*., 2008). Some of the equipment are fabric filters (Figure 11.2) and electrostatic precipitators (ESPs) (Figure 11.3). GORE developed a unique configuration of the advanced hybrid filter that promotes a synergy of combining two technologies: fabric filtration and catalytic filtration (Figure 11.4).

*Advantage:*Kim *et al*. (2000) achieved the 90–92% dioxin removal using the combined system of ESP and fabric filter. Earlier, Ergebnisse (1996) used cloth filters to achieve 73% particle-bound dioxin removal from the gaseous waste exhausting from an iron ore sintering plant.

*Disadvantage*: This is an expensive method. Technical complications arise at high temperature during the elimination of the dust from the gaseous waste of incinerators. The system also needs proper monitoring and maintenance for better results.

#### 4.1.2 Scrubber coupled with bag filter/electrostatic precipitator/spray absorber

*Principle mechanism:* Scrubbers and subsequent ESPs have been effectively used in a waste incinerator for minimizing dioxin emissions (Figure 11.5). In a spray tower, the absorbent (lime slurry) is atomized. The absorption of the gas is, to begin with, the liquid phase and then by the solid phase. In the reactor, the lime slurry blends with the

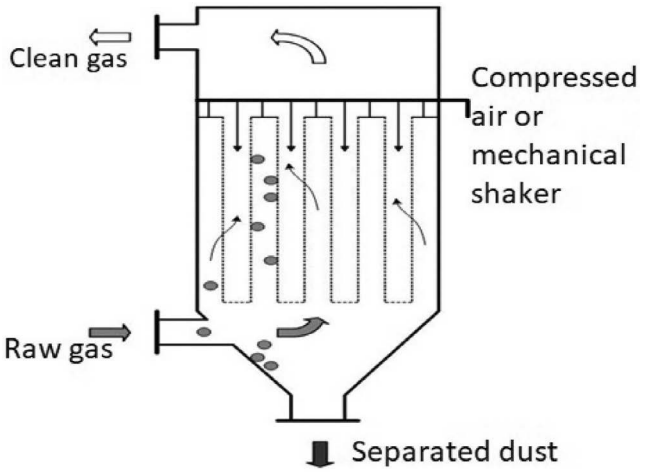


*Figure 11.2* Fabric filter.
(Source: https://emis.vito.be/en/techniekfiche/fabric-filter)

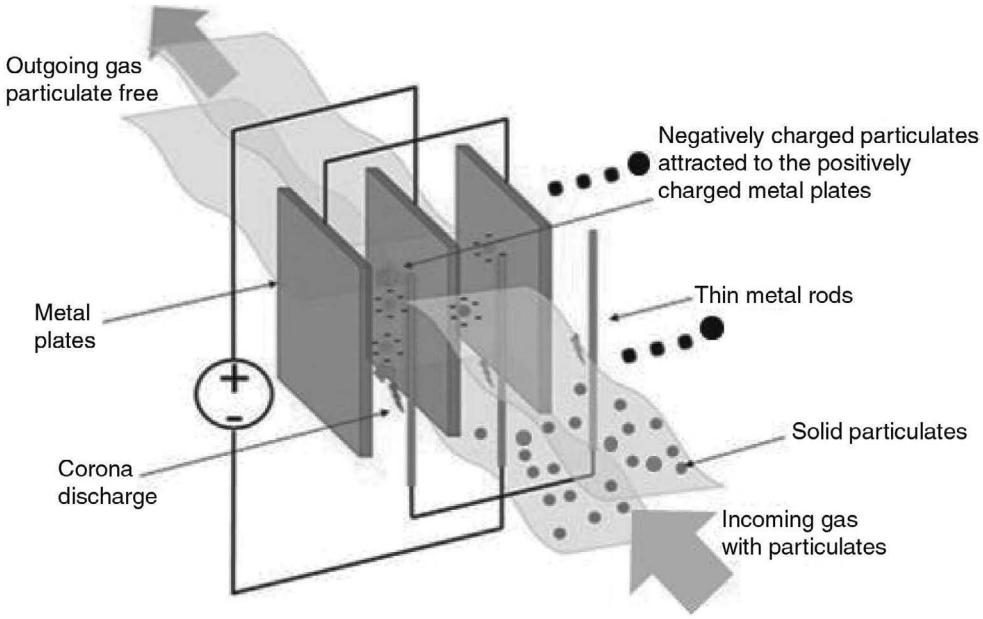


*Figure 11.3* Schematic diagram of electrostatic precipitators (ESPs).
(Source: (Becker, 2010)

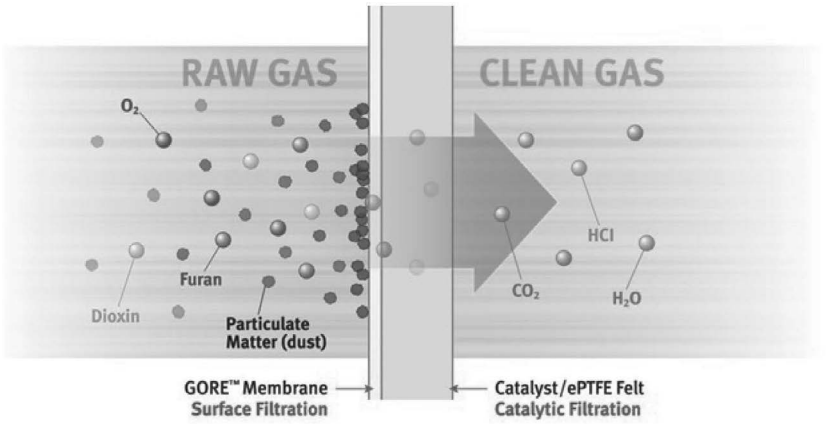


*Figure 11.4* Dioxins and furans removal technique using typical hybrid fabric filter with catalytic filtration developed by GORE™.

(Source: www.gore.com.)

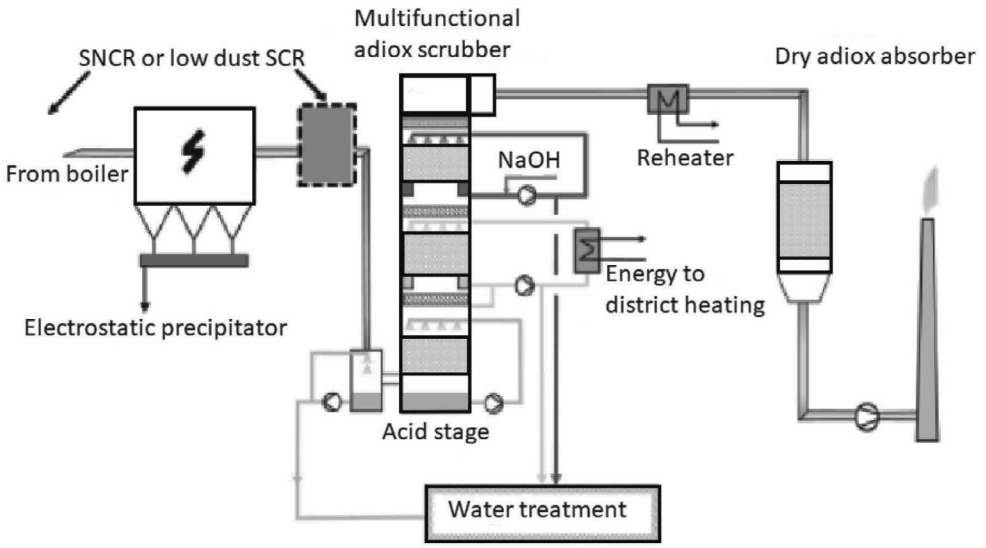


*Figure 11.5* Flue gas cleaning system comprising of an ESP, multifunctional scrubber, and dry absorber. (Source:Lindgren and Anderson, 2008)

combustion gases. The neutralizing property of the lime reduces the number of acid gas constituents (e.g., HCl and $SO_2$) in the reactor.

*Advantage:* Maier-Schwinning and Herden (1996) demonstrated that the dioxin collection efficiency could be increased by up to 90% by the supplement of coke (500 mg/m$^3$) made from bituminous coal. The fraction of acid gas constituents (e.g., HCl and $SO_2$

gas) within the reactor is minimized by the neutralization capacity of lime (Kulkarni *et al*., 2008).

*Disadvantage*: Wet scrubber is a potential source of dioxins and furans due to the "memory effect," i.e. adsorption and desorption of PCDD/Fs between the exhaust gases and the plastic filler in the scrubber (Gass *et al*., 2000; Kim *et al*., 2001).

### 4.1.3 Good combustion practice coupled with the end of pipe treatment (using bag filter and scrubber)

*Principle mechanism:* This is a two-stage system. In the first phase, the system is designed to achieve complete combustion at 850–1000 $^0$C temperature to reduce the dioxins formation. Effective "end-of-pipe treatment" (Korell *et al*., 2009; Hung *et al*., 2014) is provided in the final phase, which comprises a sequence of operations, i.e. wet scrubber, a bag filter along with carbon injection at a temperature range of 120–150 $^0$C. Carbon injection at 50 mg/Nm$^3$ which is burned in the incinerators to inhibit the PCDD/Fs generation or fixed bed adsorption system is installed for dioxin elimination (Chang *et al*., 2009; Lu *et al*., 2013).

*Advantage:* By this method, up to 99% dioxin emissions can be reduced (0.01ng TEQ/Nm$^3$). It is a cost-effective technique which involves simple engineering.

*Disadvantage*: It requires extra care and management in the selection and preparation of feedstock because it is not possible to control dioxin emissions if feedstock contains an abundance of chlorine. Also, it has high installation and running costs (Mukherjee *et al*., 2016).

### 4.1.4 Catalytic decomposition of dioxins with selective catalytic reduction (SCR)

*Principle mechanism:* The mechanism includes addition of reheated flue gas (up to 670 °C) in the catalytic reactor where oxidation of dioxins takes place in the presence of catalysts (by use of $NH_3$-SCR catalysts [$V_2O_5$-$WO_3$/TiO], $NH_3$, and urea) at high temperature (up to 410 °C) (Figure 11.6) (Boos *et al*., 1992; Chang *et al*., 2009; Dvorak *et al*., 2010; Lu *et al*., 2013). Also, silica-boria-alumina bearing oxides of Pt and Au are reported to effectively eliminate dioxins at 200 °C.

*Advantage:* Catalysts used in SCR remove dioxins up to 85% (Kulkarni *et al*., 2011). Coupling with good combustion practice can increase the efficiency of the process up to 90%. This technique eliminates intricate discard problems of residue. In 2000, Gore and associates proposed the use of a catalytic filter like "REMEDIA D/F catalytic filter system," which is found to remove 98% of dioxins.

*Disadvantage*: Regular monitoring and maintenance of the catalyst feeding process are required because excessive feeding adversely affects the reduction process. This method cannot be used in full-scale plants due to high investment and operating costs.

### 4.1.5 Addition of sulfur compounds (using oxidation and reduction processes with recycling method)

*Principle mechanism:* In this method, formation of PCDD/Fs are reduced due to sulphur compounds like pyrite and ammonium sulphate as they turn metal catalysts into sulfates (by oxidation process) or sulphides (by reduction process) (Figure 11.7) (Xie *et al*., 2000; Liu *et al*., 2015). Sulfur compounds can also be recycled and thus reused.

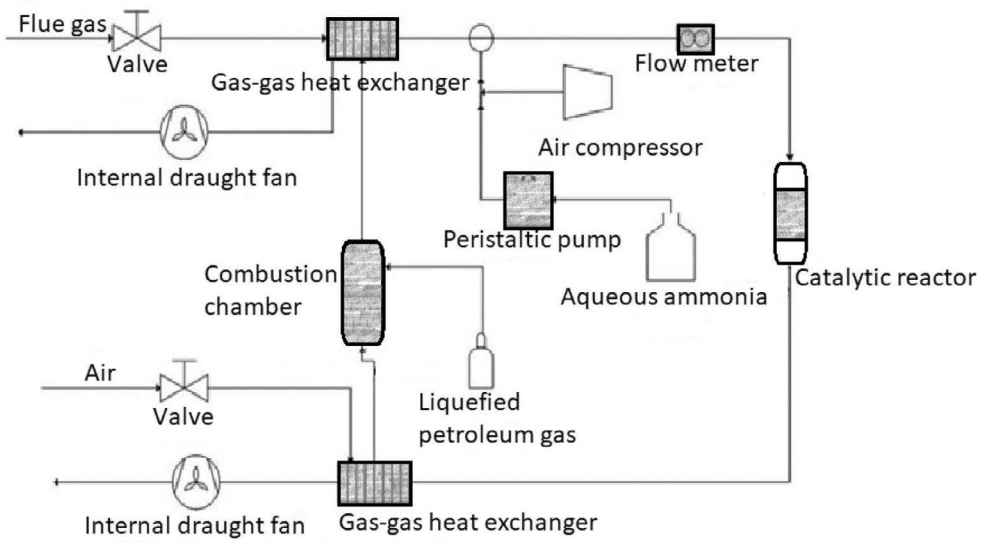


*Figure 11.6* Flow diagram showing PCDD/PCDFs inhibition by the aid of $V_2O_5$-$WO_3$/$TiO_2$ catalysts. (Source: Vermeulen *et al*., 2014)

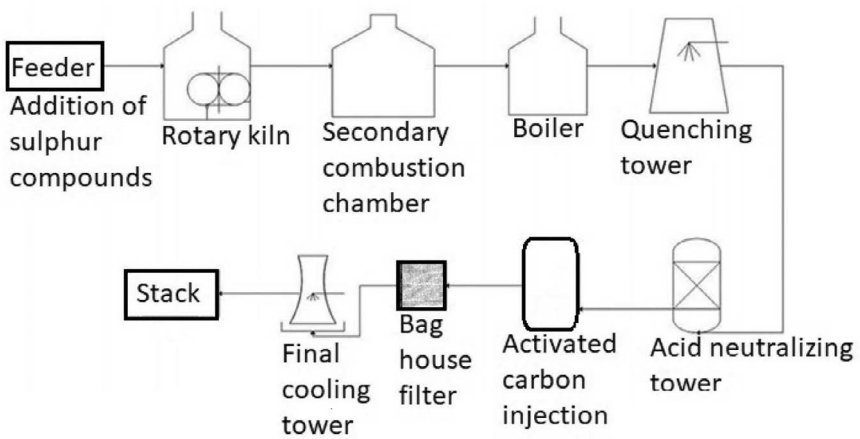


*Figure 11.7* Flow diagram of sulfur dioxide circulation system.
(Source: Liu *et al*., 2015)

*Advantage:* Samaras *et al*. (2000) reported the 98.3% PCDD/F removal efficiency to near about 0.1ng TEQ/$Nm^3$ when combined with pyrite. This method is economical due to the recycling of the inhibitor (Liu *et al*., 2005).

*Disadvantage*: Poisoning of metal catalyst surfaces due to reduction or oxidation process is a drawback.

### 4.1.6 Addition of nitrogenous compounds as inhibitors

*Principle mechanism:* This method involves complex reaction with metal catalysts forming a strongly bonded organo-metallic nitride complex leading to the irreversible deactivation of the catalytic site along with restricting the chlorination to inhibit the formation of dioxins (Luna *et al*., 2000). Inhibitors like mono-ethanolamine (MEA), ethanolamine, urea, thiourea, ammonia, triethanolamine, and di-methylamine are used in this technique (Tuppurainen *et al*., 1999; Lin *et al*., 2015). A waste incinerator using thiourea is illustrated in Figure 11.8.

*Advantage:* 95% removal efficiency can be achieved by nitrogen-containing compounds (Mukherjee *et al*., 2016). No poisoning of the catalytic surface takes place. Deactivation of the active sites of catalysts occurs.

*Disadvantage*: A high combustion temperature is required in the process.

### 4.1.7 Activated carbon adsorption

*Principle mechanism:* The process involves two phases: In the first phase, PCDD/Fs are eliminated by the inoculation of activated carbon into the exhaust stream and in the second phase, a fabric filter or electrostatic precipitator are positioned downstream to eliminate activated carbon particles and residues (Figure 11.9) (Liu *et al*., 2012). Depending on the type of adsorbents, the adsorption process is of three types: entrained flow, fixed-bed, and moving-bed processes.

*Advantage:* Activated carbon has high PCDD/Fs adsorption efficiency. It is commonly used in the incineration plant (Everaert *et al*., 2003; Karademir *et al*., 2004; Mori *et al*., 2005). This is a simple and convenient technology.

*Disadvantage*: Cooled exhaust gases are injected with activated carbon to avoid the de novo synthesis, and the process consumes an abundance of activated carbon, making it an expensive technique (Everaert *et al*., 2003).

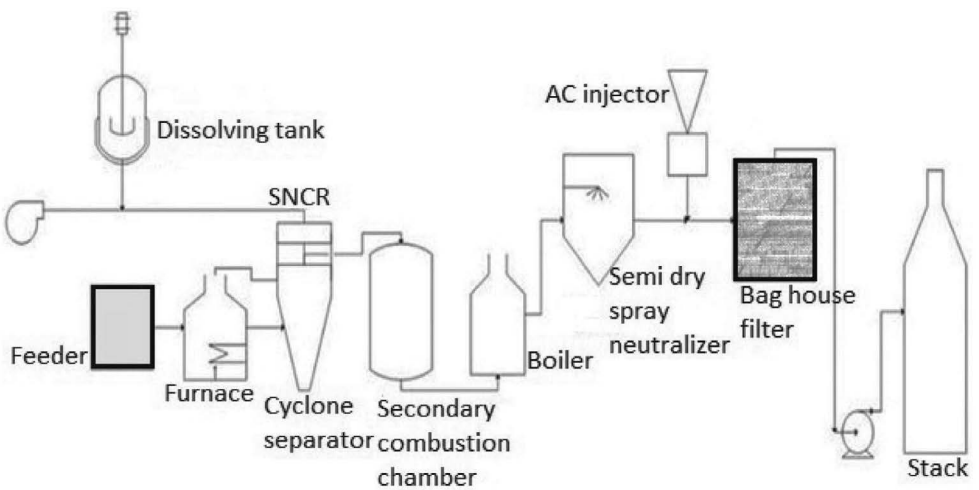


*Figure 11.8* Flow diagram of the waste incinerator that uses thiourea.
(Source: Lin *et al*., 2015)

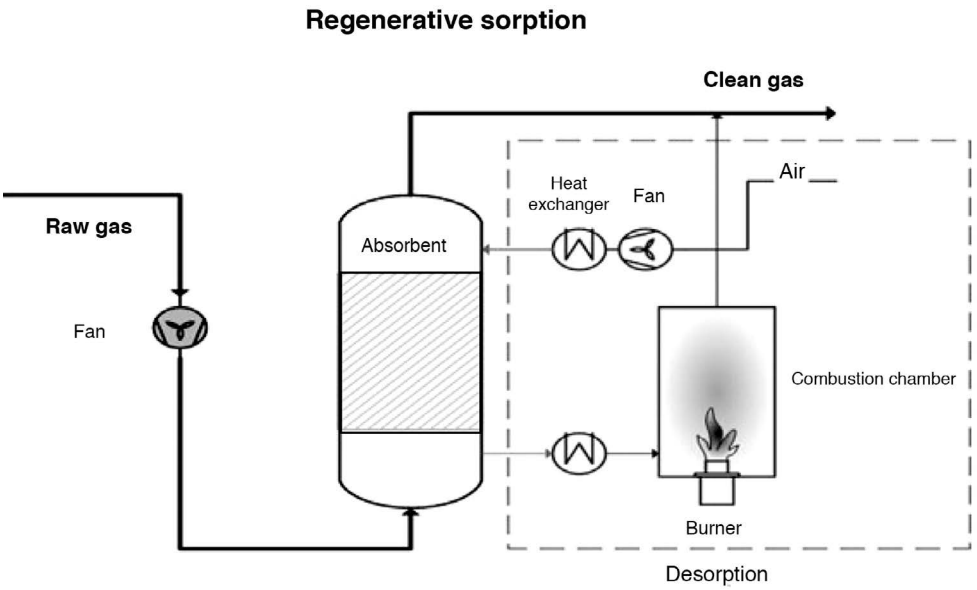


*Figure 11.9* Activated carbon adsorption with regeneration processes.
(Source: https://emis.vito.be/nl/node/19457)

### 4.1.8 Sorbent or flow injection process

*Principle mechanism:* In sorbent or flow injection process, fine-grained coke stemming from anthracite or bituminous coal blended with lime, limestone, or inert material is injected into the waste gas flow at 120 °C (Figure 11.10) (Kulkarni *et al*., 2008). As a result, the material becomes homogeneously suspended in the flow and precipitates in the form of a layer on the cloth filter. The added inert material assists in the adsorption process and avert coke ignition (Cudahy and Helsel, 2000). Abad *et al*. (2003) proposed natural or synthetic zeolites as a good alternative.

*Advantage:* 99% of dioxins can be removed by this process. This technique is frequently used in waste incineration plants for the removal of dioxins, $SO_2$, HCl, and HF (Kulkarni *et al*., 2008).

*Disadvantage:* The drawback is the requirement of a high amount of inert material in the technique and a substantial amount of residue generated from the system.

### 4.1.9 The fluidized-bed process with adsorbent recycling

*Principle mechanism:* In this method, the flue gas enters through the grate from the bottom and creates a fluidized bed of coke stemming from bituminous coal and inert material, such as lime/limestone, at a temperature of 100–120 °C (Figure 11.11). The adsorbent is segregated from the flue gas and collected in a dust collector from where this is re-circulated to the fluidized bed (Kulkarni *et al*., 2008).

*Advantage:* Advantageous conditions for mass and heat transfer and greater residence times of adsorbent in the system result in better utilization of sorbent (Liljelind *et al*., 2001;

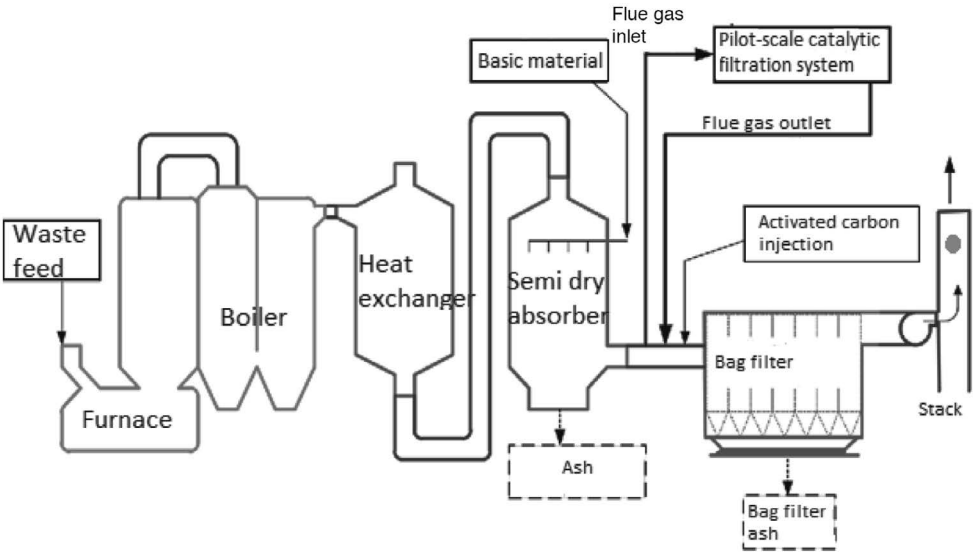


*Figure 11.10* Sorbent or flow injection process.
(Source: Hung *et al*., 2014)

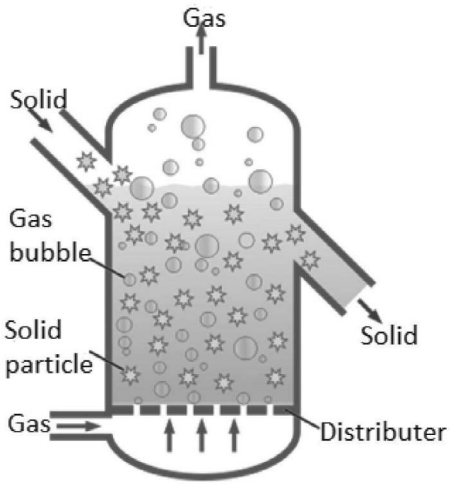


*Figure 11.11* Fluidized-bed process with adsorbent recycling.
(Source: http://civilenggseminar.blogspot.com/2014/11/activated-carbon-adsorption.html)

Shiomitsu *et al*., 2002). The adsorbent can be reused several times, making it possible to remove other acid components such as HCl, HF, and $SO_2$.

*Disadvantage:* Significant disadvantage is still known.

### 4.1.10 Fixed-or moving-bed process

*Principle mechanism:* The same adsorbent is used as that of the fluidized-bed technique, but it is slightly different; the coke gradually moves from top to bottom, while the waste gas flows in reverse direction (Figure 11.12). The difference between fixed and moving-bed processes is that in the earlier, the bed of activated coke is static during adsorption, and the used coke is withdrawn and replaced by fresh coke. In moving-bed reactors, the coke bed runs continuously (Fell and Tuczek, 1998).

*Advantage:*Karademir *et al*. (2004) reported that the 99% removal efficiency could be attained by this method. For an effective exchange of matter, the period is 10 times longer than the fluidized bed or flow injection techniques (Fell and Tuczek, 1998).

*Disadvantage:* No major shortcoming is reported.

### 4.1.11 Electron irradiation processes

*Principle mechanism:* Electron irradiation (Figure 11.13) is a novel process for dioxin remediation in the flue gas, which involves gas-phase dioxin degradation by OH radicals. These OH radicals are formed by the action of ionizing radiation on gas macro components (Gerasimov, 2001). Electron beam radiation, together with hydrated lime, can eliminate HCl, NOx, $SO_2$, and chlorine compounds present in exhaust gases. The electron beam

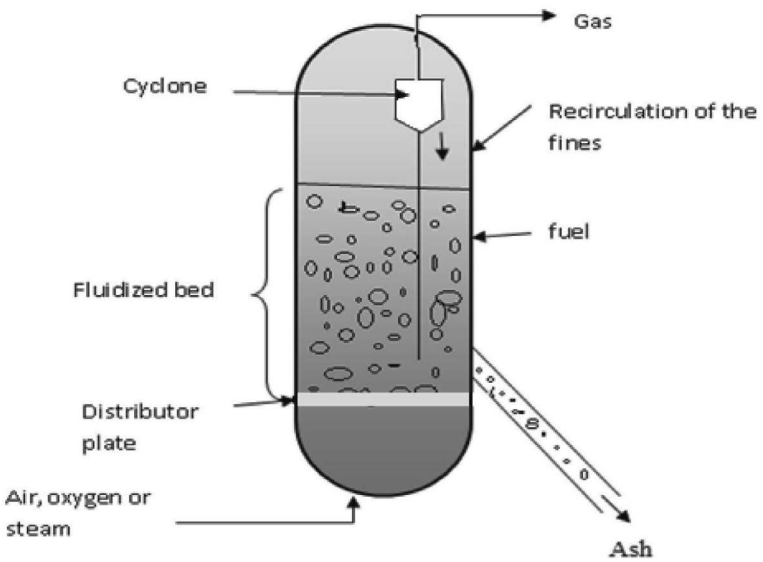


*Figure 11.12* Fixed or moving-bed process.

(Source: http://civilenggseminar.blogspot.com/2014/11/activated-carbon-adsorption.html)

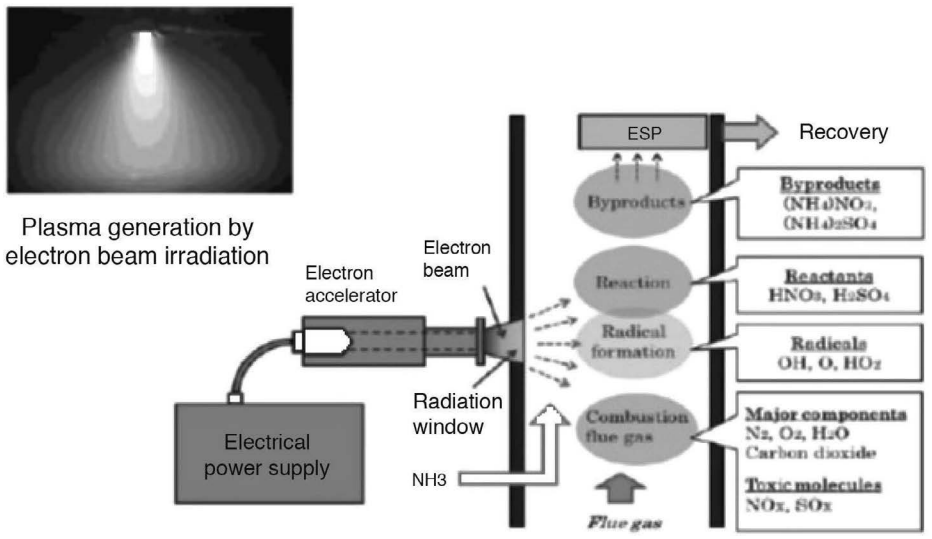


*Figure 11.13* Electron irradiation processes for dioxin removal.
(Source: Licki *et al.*, 2013)

disintegrates the noxious chlorine compounds into innoxious organic acids, like acetic acid, chloroacetic acid, and formic acid.

*Advantage:* The benefits of this process include low-energy consumption and improbable secondary pollution due to the direct decomposition of dioxins. The process does not require temperature control. It is a straightforward process which can be easily installed in existing incinerators. Moreover, electron beam radiation can manage massive volumes of exhaust gases.

*Disadvantage:* No disadvantage is reported so far.

### 4.1.12 Hybrid technology (Plasma technology and catalysis)

*Principle mechanism:* Plasma technology is a novel technology which may generate some harmful by-products in the treatment procedure, which are overcome by a combination of plasma and catalysis (Figure 11.14) (Zhou *et al*., 2003; Hung *et al*., 2010; Liu *et al*., 2012). The combination of plasma and catalysis significantly reduces the operating temperature of the hybrid system along with the elimination of dioxins by numerous active particles produced from plasma.

*Advantage:* This technology has a very high PCDD/Fs removal efficiency.

*Disadvantage:* Hybrid technology is quite expensive.

## 4.2 *Removal of dioxins from soil/sediments/wastes/fly ash*

Soil contamination by dioxins and furans (PCDD/Fs) is a serious problem in the industrial expanse. Their remediation includes excavation (for ex-situ) followed by treatment methods like physical, biological, chemical, and thermal methods then finally, disposal

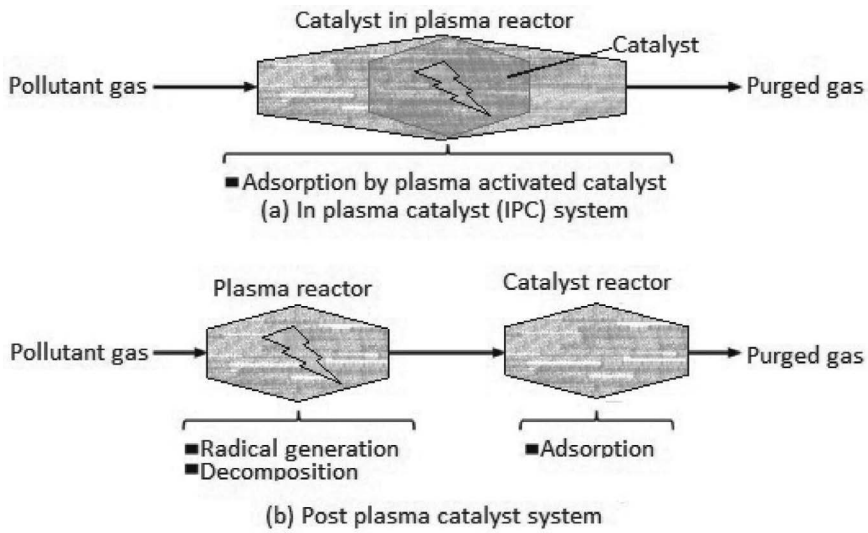


*Figure 11.14* Hybrid technology; Plasma technology + catalysis. (Source: Matsumoto *et al*., 2012)

in suitable landfilling. Some of these treatment methods are also applied to in-situ soil treatment. The PCDD/Fs are very resistant to biodegradation processes and owing to their long half-lives, i.e. 3.2 to 5.8 years, they are quickly accumulated in the environment (Wang *et al*., 2011). 2,3,7,8-TCDD has a half-life of about ten years in soil (Jones and Sewart, 1997). The solubility of dioxins is very low in the water due to their non-polar and hydrophobic nature and high octanol/water partition coefficients (ATSDR, 1998). PCDD/Fs are readily adsorbed by suspended particles present in the water or can be settled by sedimentation, owing to their hydrophobic properties and densities (Kim *et al*., 2009).

The incineration processes of municipal solid waste, medical wastes, and hazardous sewage sludge produce condensed solid residues or cake, which is termed as fly ash, which contains dioxins (100–5000 ng/kg).

Under the environmental protection legislation in various countries, municipal solid waste incineration fly ash is categorized as hazardous material, which requires further treatment before their disposal in landfills or release into the atmosphere. A variety of treatment methods are applied to destroy dioxins in fly ash, with most of the methods similar to those used for soil/sediment/waste treatment methods. Thermal treatments are the most promising technology, which is widely practiced at industrial scale to treat PCDD/Fs laden soils. Currently, thermal desorption is practiced at full-scale for the treatment of soils polluted with organic compounds, although the treatment is costly (Dai *et al*., 2013; Lundin *et al*., 2013; Zhao *et al*., 2013; Tritz *et al*., 2014). The remediation technologies being used to remove dioxins from soils/sediments/wastes/fly ash are discussed below.

### 4.2.1 Thermal desorption (TD) process (using vaporization and degradation)

*Principle mechanism:* Heat is applied to the contaminated soil/wastes/fly ash for volatilization/ desorption of hydrocarbons, which are then carried away by a vacuum or sweep gas

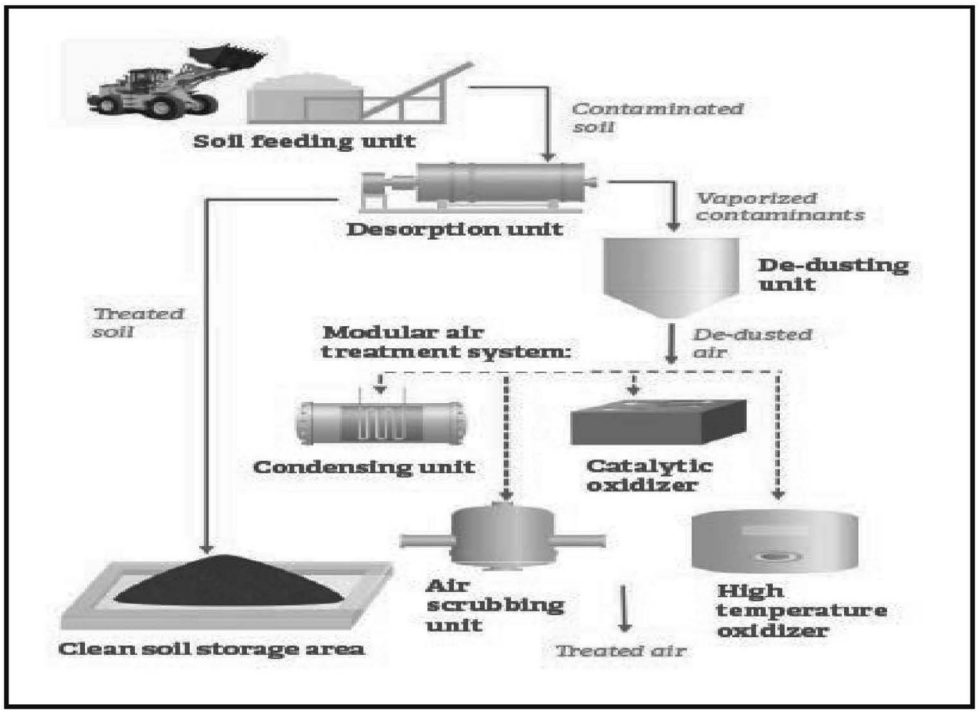


*Figure 11.15* Thermal desorption process.
(Source: http://dekonta.cz/wp content/uploads/2016/12/Dekonta_letak-A4_IFAT_Plant_web.pdf)

and finally treated by carbon adsorption processes or incineration (Figure 11.15) (Riser-Roberts, 1998; Baker and Kuhlman, 2002; Khan *et al*., 2004; Vidonish *et al*., 2016b). There may be low-temperature thermal desorption (LTTD, 100–300 °C) or high-temperature thermal desorption (HTTD, 300–550 °C). It may be in-situ or ex-situ thermal desorption. More than 90% reduction of the PCDD/Fs concentration was realized by remediating polluted soil at 280 °C and addition of non-sized zero-valent iron (nZVI) to the soil, ensuring almost complete removal of PCDD/Fs in the gas phase.

*Advantage:* TD is efficient for polychlorinated dibenzo-p-dioxins (PCDDs) removal up to 99% (Stegemeier and Vinegar, 2001). No off-site transport costs and no long-term liability exist. Treated soils and sediment can be used onsite.

*Disadvantage:* Highly toxic dioxins may be formed in the thermal desorption process at low temperature below 400 °C, e.g., significant amount of PCDF formation occurs due to loss of ortho-$Cl_2$, HCl, ortho-$H_2$, and dechlorination if soil/sediments contaminated with PCBs. Excavation of soil or sediment is obligatory, which may harm flora and fauna. Multiple treatment piles or cells are required for the sequencing of treatment, which need appropriate equipment and intensive labor resources. Thermal techniques, when applied to wet clay and silty soils, then consume more fuel, raising the treatment cost to remove contaminants.

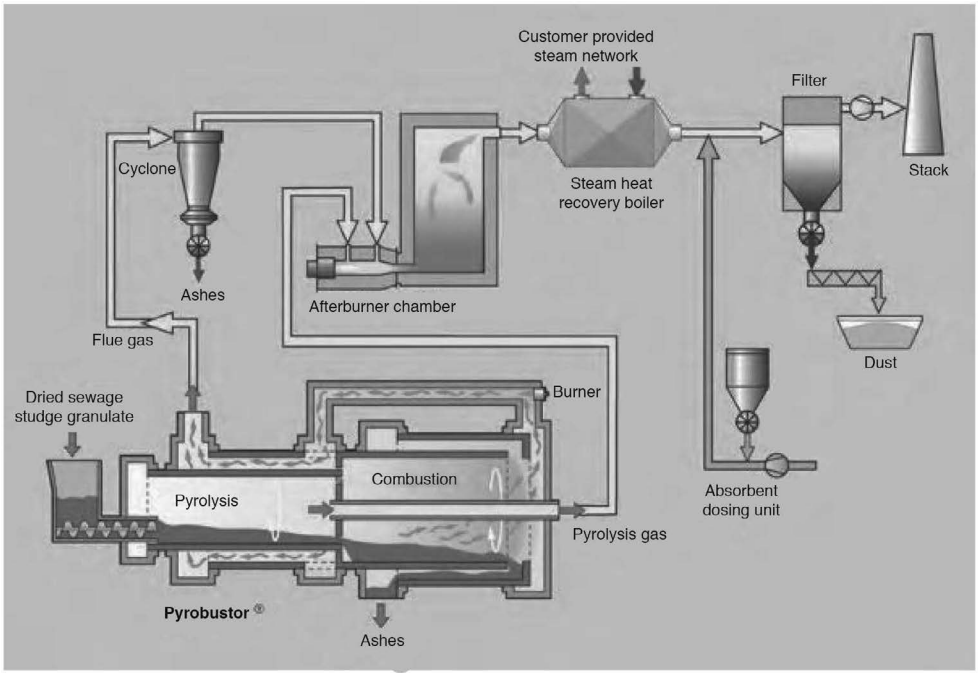


*Figure 11.16* Pyrolysis method (Godheja *et al*., 2016).

### 4.2.2 Pyrolysis (for hazardous wastes)

*Principle mechanism:* The pyrolysis method (Figure 11.16) involves the heating of impacted soils at temperature 400–1200 °C in inert atmospheres (Guemiza *et al*., 2017).

*Advantage:* Pyrolysis has a removal efficiency of PCDD/Fs greater than 99.99% in contaminated soil. It preserves nutrients and soil properties that are usually lost during the incineration of soil. Char produced during treatment increases the carbon content of the soil. The volatile products formed by pyrolysis can be reused similar to the thermal desorption method.

*Disadvantage:* 2,3,7,8-TCDD is significantly formed if lower retention time is given for destruction (Guemiza *et al*., 2017). The short reaction time may give rise to higher TEQ value of PCDD/Fs due to incomplete dechlorination. Pyrolysis is an expensive method and requires maintenance for inert conditions. This method has 100% removal efficiency of PCDD/Fs at a temperature between 500 and 950°C, but numerous transitional species are formed, such as polyaromatic hydrocarbons, eight benzofuran, and other volatile organic compounds, increasing air pollution.

### 4.2.3 Photocatalysis (using photodegradation and catalysis processes)

*Principle mechanism:* Semiconductor films, such as $TiO_2$, ZnO, $Fe_2O_3$, ZnS, CdS, are used for UV or solar light-induced photocatalytic degradation of dioxins (Diebold *et al*., 2003; Kulkarni *et al*., 2011). In the photocatalysis process (Figure 11.17), light is used to induce conduction band (CB) electrons and valence band (VB) holes (e− and h+),

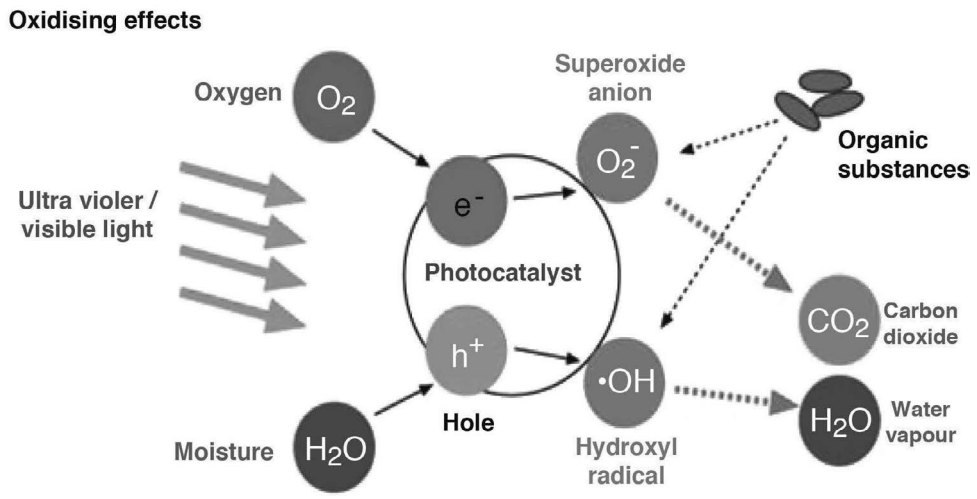


*Figure 11.17* Photocatalysis processes.
(Source: http://primustech.com.sg/pmi-titanium-dioxide-photocatalyst/)

which starts redox reactions on semiconductors (Kulkarni *et al*., 2011). A low-pressure ultraviolet mercury lamp is used with active catalysts (e.g., hydroxyl radical, heat-activated persulfate [HAP], $SnO_2$, $TiO_2$, gold doped zeolites, and silver doped zeolites.) for the photodegradation of dioxins for ex-situ process. The in-situ method includes penetration of UV light from the sun into contaminated soil along with organic solvent; for instance, Isosaari *et al*. (2004) used vegetable oils to increase the solubility of organic compounds for photodegradation.

*Advantage:* Photocatalysis degrades PCDD/F in a short amount of time. Wu *et al*. (2005) demonstrated the photocatalysis of dioxins like 1,2,3,6,7,8-HxCDD and 2,3,7,8-TCDD under $TiO_2$, $SnO_2$, and ZnO catalysts. Furthermore, Samara *et al*. (2017) reported that silver modified zeolite degrades 2,3,7,8-TCDD up to 86% at 254 nm UV irradiation for 6 h. 60% acetonitrile/water solutions of 2,7-DCDD and 1,2,7,8-TCDD at 300 nm UV can be photodegraded.

*Disadvantage:* Photocatalysis is an expensive method and a slow process of dioxin removal. Easy photo corrosion, easy agglomeration, the low conversion rate of solar energy, and problematic post-separation of the inorganic catalysts may limit the extensive applications. Proper maintenance and monitoring of the system are required. The dioxin removal efficiency of the process is slower than other methods and application is restricted by the low UV ray penetration into lower soil depth for the in-situ method and the form of molecules present.

### 4.2.4 Biodegradation

*Principle mechanism:* In biodegradation, microorganisms are used (bacteria and fungi, e.g., lignocellulosic substrate, *Lentinulus edodes, Pleurotus ostreatus, Stropharia rugosoannulata, Agaricus bisporus*) for dechlorination under the anaerobic and reducing environment

or for deoxygenation under the aerobic environment to break down PCDD/Fs into simple compounds, i.e. $H_2O$, $H_2$, $CO_2$, $CH_4$, and chloride or to convert into microbial cellular material (INSERM, 2000; Johnson, 2008; Kulkarni *et al*., 2011; Liu *et al*., 2014; Chen *et al*., 2013).

*Advantage:* White rot fungus has a degradation value of 40% (TCDD) to 76% (HxCDD) for PCDDs and 45% (TCDF) to 70% (HxCDF) for PCDFs. Once the degradation is complete, the microbial population is reduced due to the absence of their food source.

*Disadvantage:* Biodegradation is a slow process. The high efficiency is limited to lower chlorinated dioxins. Climatic conditions and biotic composition of the soil are the limiting factors of the process (CCME, 2002). The bioremediation process is very sensitive to the temperature, moisture content, and nature of the contaminants to be remediated and the morphology/geology of the contaminated site. The peridechlorination process (elimination of chloride atoms in the position 1,4,6,9) may elevate the toxicity of PCDD/Fs molecules, forming the 2,3,7,8-TCDD/F molecules (Kuokka *et al*., 2014).

### 4.2.5 Hybrid technology (used with adsorption and photocatalytic degradation processes)

*Principle mechanism:* This remediation process involves the use of hybrids like metal-organic frameworks (MOFs) – a novel category of functional inorganic-organic hybrid materials (Figure 11.18).

*Advantage:* Hybrid materials provide a super-large surface area and pore volume. It has versatile applications like in adsorption, photocatalysis, separation, and electrochemistry processes. The hybrid material can be regenerated after use.

*Disadvantage:* Except few, most of the MOFs in the presence of water are unstable, ensuing in low recovery, and it may create pollution due to the leaching metal.

### 4.2.6 Incineration (for soil, hazardous, and municipal wastes)

*Principle mechanism:* In the incineration unit (Figure 11.19), PCDD/Fs are converted into simple and non-toxic molecules in the presence of oxygen at high temperatures (600–1600° C) (Shearer, 1991; Barnes *et al*., 2002; Vidonish *et al*., 2016a). The exhaust gases are

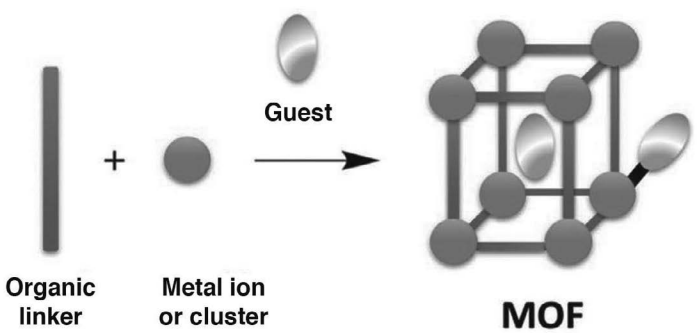


*Figure 11.18* Metal-organic frameworks.
(Source: Calbo *et al*., 2019)

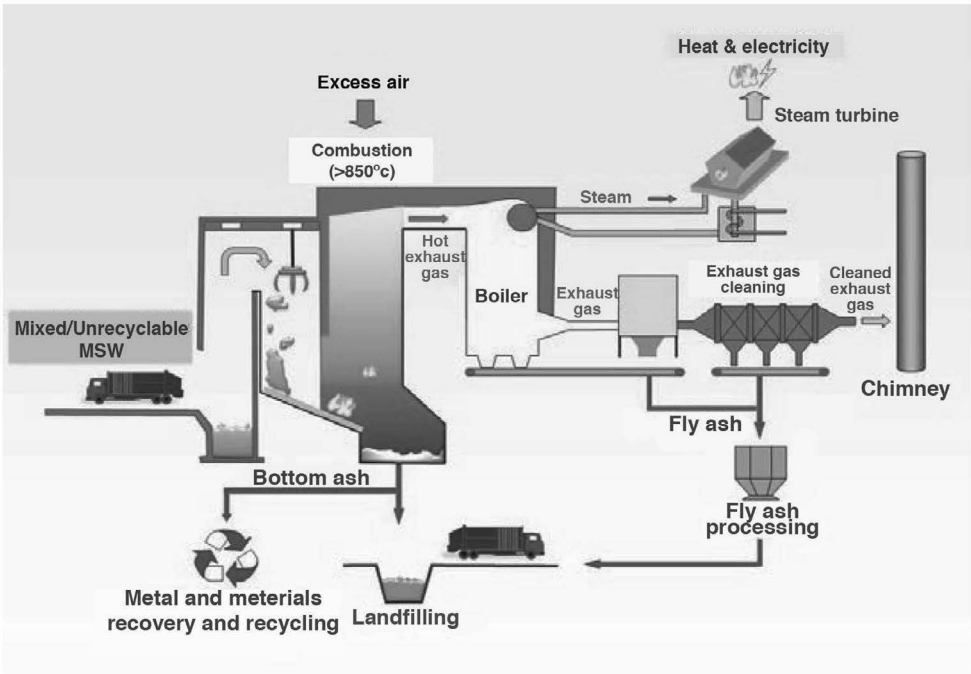


*Figure 11.19* A typical flow chart of the incineration unit.
(Source: www.epd.gov.hk/epd/english/environmentinhk/waste/prob_solutions/WFdev_IWMFtech.html)

filtered using electrostatic precipitators/scrubbers/baghouses to prevent air pollution and soil contamination (Shearer, 1991; Morselli *et al*., 2008). Then the fly ash thus collected is treated separately.

*Advantage:* Removal efficiency of incineration is more than 99%, which removes a wide range of target contaminants. High-temperature combustion provides destruction of contaminants, and this technique is suitable for energy recovery.

*Disadvantage:* It is the most expensive thermal technology. It needs further treatment of flue gas. Inflowing oxygen levels are upheld at 10% for VOCs combustion. The high temperature decomposes the organic matter present in soils, thus limiting the reuse of treated soil.

### 4.2.7 Vitrification

*Principle mechanism:* The vitrification process (Figure 11.20) uses exceptionally high temperatures, i.e. 1600–2000 °C, to melt and fuse impurities and soil into a glass-like solid (Vidonish *et al*., 2016b). Crystallization is prevented by swiftly cooling the molten soil and impurities. Consequently, stable glass is formed from non-volatile materials (Shearer, 1991). The volatile substances desorb and are treated as a waste gas stream similar to the case of pyrolysis. Vitrification is effective for more toxic materials (Yeung, 2010).

*Advantage:* This method can be applied to soils with various organic contaminants. Vitrification is mainly used to treat hazardous wastes.

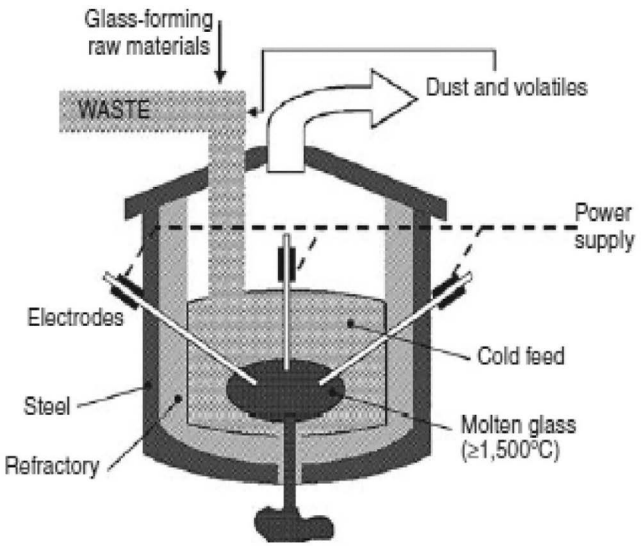


*Figure 11.20* Vitrification processes for hazardous-waste treatment.
(Source: Bernardo *et al*., 2012)

*Disadvantage:* Vitrification reduces 20% – 40% of soil volume (Shearer, 1991; Hamby, 1996). Thus, additional backfill soil needs to be added after excavation. To control air pollution, additional technologies are required like baghouses, electrostatic precipitators, and incineration.

### 4.2.8 RFH or microwave heating (for hazardous wastes and biomedical wastes)

*Principle mechanism:* In radio frequency heating **(**RFH), or microwave heating, heat is transferred on a molecular level by applying an electric field on electric dipoles in soil, pollutants, and water (Figure 11.21). Microwave treatment is done using microwave absorbing agents like powder-activated carbon (PAC) (Figure 11.22).

*Advantage:* RFH is equally effective in soil with heterogeneous properties (Price *et al*., 1999). The degradation efficiency of the PCDD/Fs in the soil is found to be 99.6% at microwave incident power of 2100 W for 7 min. Degradation efficiency of PCDD/Fs in hospital solid waste incinerator fly ash is reported to be 91.9% at a microwave incident power of 1800 W for 7 min.

*Disadvantage:* Treatment duration is in order of days and differs according to the pollutant, temperature, and whether the RFH is used as a stand-alone or auxiliary technology.

### 4.2.9 Non-thermal nanosecond plasma method (for dioxin-contained fly ash/flue gas)

*Principle mechanism:* Detoxification of dioxin contained fly ash or flue gas is completed using positive pulse discharge due to which surface chemical conversion of dioxin-contained fly ash is achieved as illustrated in Figure 11.23 (Zhou *et al*., 2003).

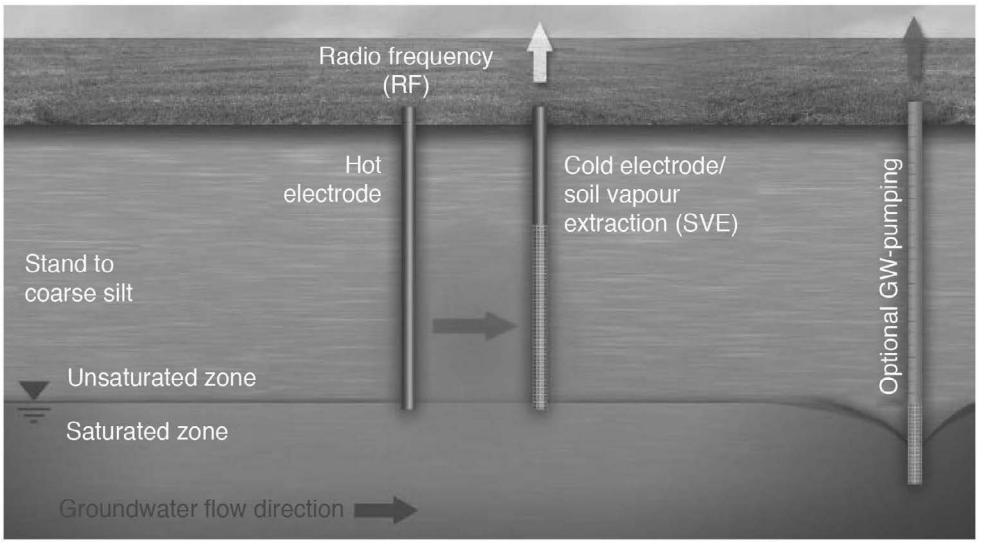


*Figure 11.21* Schematic diagram of the radio frequency sub-surface heating.
(Source: www.reconsite.com/fileadmin/dateien/Publikationen/ISTT_Guidelines_FINAL_PRINT.pdf)

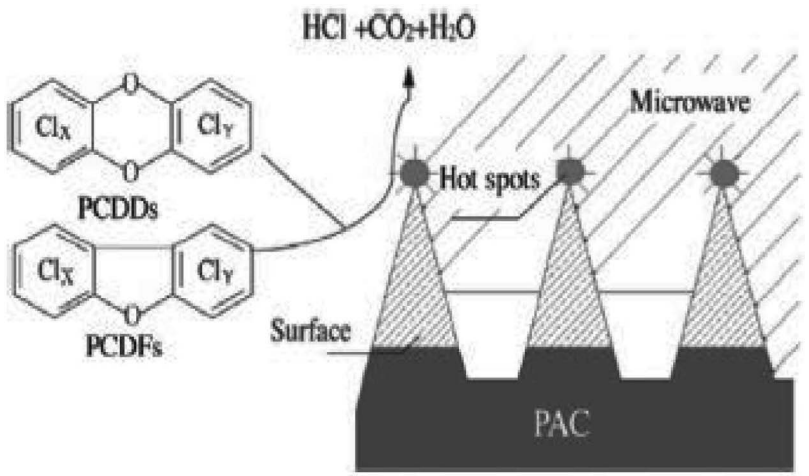


*Figure 11.22* Decomposition mechanism of PCDD/Fs by the "hot spots" form of powdered activated carbon (PAC) surface, during microwave heating.
(Source: Wei *et al*., 2017)

*Advantage:* Highly toxic compounds can be more easily destroyed by this process. The degradation efficiency of 2,3,7,8-TCDD is up to 81%. It effectively and economically removes dioxins at very low concentrations under ambient temperature conditions, and little maintenance is required. It does not need auxiliary fuel, and there are no disposal problems. Also, there is no risk of toxicity by sulfur or halogen-containing compounds.

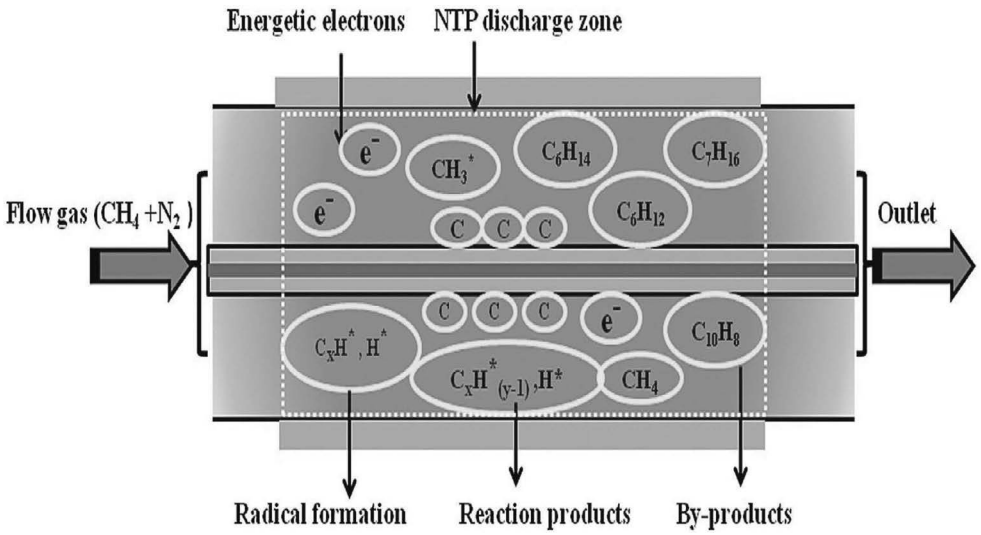


*Figure 11.23* Non-thermal nanosecond plasma method.
(Source: Mustafa *et al.*, 2018)

*Disadvantage:* It is an expensive method, and degradation efficiency needs to be improved for the treatment of other dioxins.

### 4.2.10 Successive self-propagating sintering process (SSPSP)

*Principle mechanism:* Sintering process uses carbonaceous material, e.g. charcoal B at temperature 800–1000 °C and air drawn through the sintering bed by a pump (Figure 11.24) (Zhao *et al.*, 2015). The exhaust gas is drawn through the bed and passes through a sequence of traps before their release into the environment.

*Advantage:* Removal efficiencies for PCDD/Fs from sediment is found to be more than 99.9% while for the soil it is 52%. It is a cost-effective and novel technology which can be further improved in the future to meet efficient removal.

*Disadvantage:* It needs further improvement for increasing efficiency for soil treatment. This system also requires periodic monitoring.

### 4.2.11 Leaching processes (soil washing technique)

*Principle mechanism:* The leaching process involves extraction or the solubilization of dioxins using numerous leaching agents, for example, chelating agents, inorganic or organic acids, oxidizing agents, or solvents (Figure 11.25). Granulometric sorting is done to separate fine soil particles from coarser particles present as PCDD/F and are adsorbed onto the finely grained soil due to high affinity. According to the USEPA (1990), the ex-situ soil washing will be efficient for both polar solvents (acetone, acetonitrile, methanol, and ethanol) and non-polar solvents (dichloromethane, hexane).

*Advantage:* Leaching processes can be applied in-situ or ex-situ. Leaching processes are faster and more promising for recalcitrant organic compound removal than biological

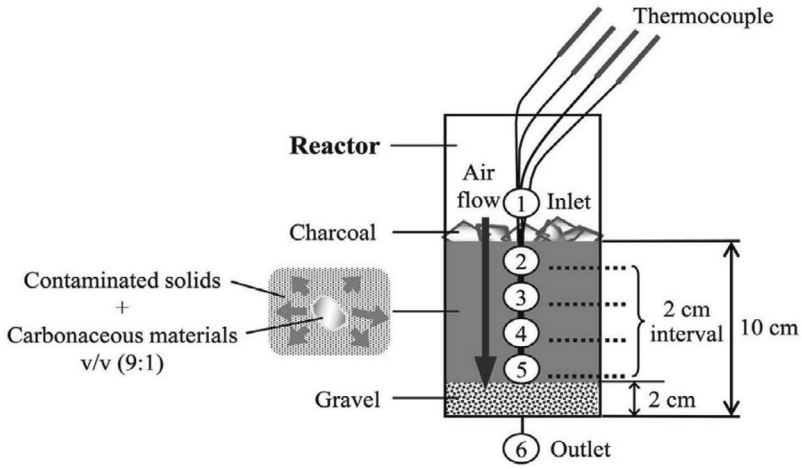


*Figure 11.24* Reactor used in Successive self-propagating sintering process (SSPSP) for dioxin removal. (Source:Zhao *et al*., 2015)

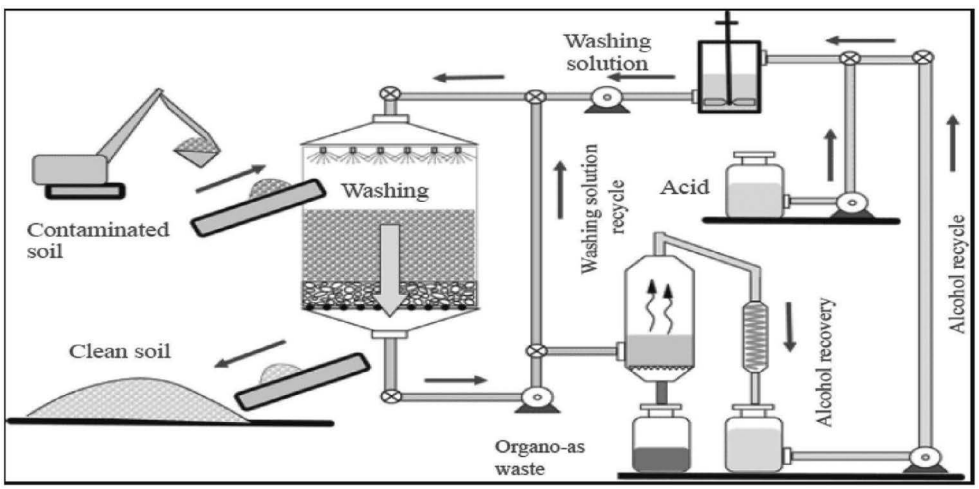


*Figure 11.25* Ex-situ leaching processes for dioxin-contaminated soil treatment.
(Source: Godheja *et al*., 2016)

treatments. Jonsson *et al*. (2010) demonstrated the removal of 80% of PCDDs and 98% of PCDFs from soil using an ethanol solution (75%). Similarly, Sahle-Demessie *et al*. (2000) removed 95% of PCDD/F in the presence of dimethyl ether. Also, Mitoma *et al*. (2004) reduced up to 98–100% of PCDD/F and PCB in the soil at room temperature, using metallic calcium in ethanol.

*Disadvantage:* The leaching process is currently not in use at an industrial scale due to the lack of organic pollutant removal and costly operation. Moreover, granulometric sorting reduces soil volume and thus increases rehabilitation costs.

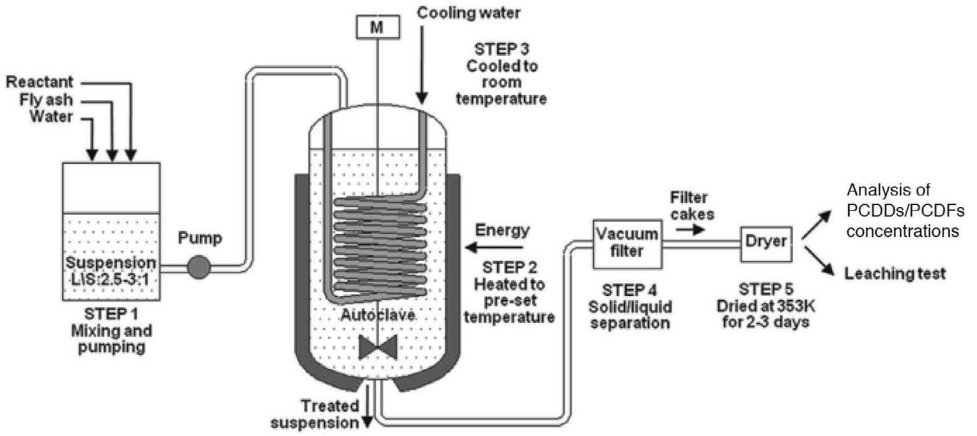


*Figure 11.26* Process scheme of the hydrothermal process.
(Source: Hu *et al*., 2012)

### 4.2.12 Hydrothermal treatment

*Principle mechanism:* Fly ash, when reacting with suitable additives, produces novel cementitious materials (De Rojas *et al*., 1993; Jing *et al*., 2007). A hydrothermal treatment (Figure 11.26) is a physicochemical technique based on the relation between temperature, relative humidity, and time (Kulkarni *et al*., 2008). Fly ash put into water/solution (e.g., NaOH containing methanol) at high temperature and pressure for hydrothermal treatment proved to be highly effective for dioxin decomposition.

*Advantage:* This treatment technology has a high efficiency. Kulkarni *et al*. (2008) reported that fly ash containing 1100 ng/g of dioxins subjected to hydrothermal treatment using NaOH containing methanol solution at 300 °C for 20 min was found to have 0.45 ng/g of dioxins left. The process is better than pure thermal treatment at an identical temperature. The treated fly ash can be used in the cement industries.

*Disadvantage:* No significant disadvantage is reported.

### 4.2.13 Supercritical water oxidation (SCWO)

*Principle mechanism:* Supercritical water oxidation (Figure 11.27) is a waste treatment technique applying supercritical water, which occurs as a phase above the critical temperature (647.3 K) and critical pressure (22.12 MPa) in the presence of oxidizers such as air, $O_2$ gas, and pure $H_2O_2$ (Sako *et al*., 1997). The hybrid process using supercritical fluid ($CO_2$), adsorption (activated carbon), and degradation by SCWO caused degradation of dioxins in fly ash (Sako *et al*., 2004).

*Advantage:* 99.7% decomposition yield of dioxins can be achieved with the use of supercritical water and $H_2O_2$ (Sako *et al*., 1999).

*Disadvantage:* Major downside is not reported.

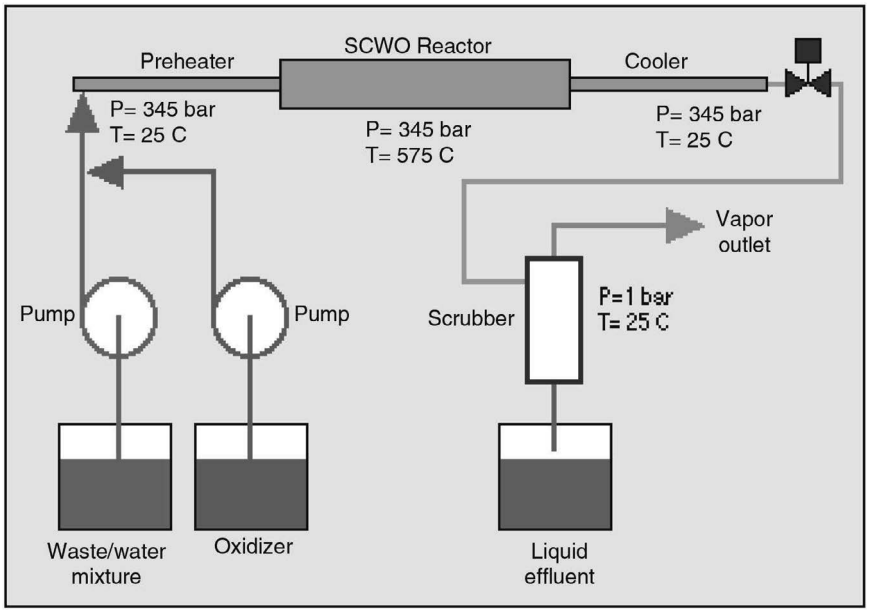


*Figure 11.27* Schematic diagram of supercritical water oxidation (SCWO) waste treatment system. (Source: www.turbosynthesis.com/summitresearch/sumscw1.htm#anchor2456210)

### 4.2.14 Radiolytic degradation (soil/wastewaters/fly ash)

*Principle mechanism:* Ionizing radiation in the form of high-energy electron beams and gamma rays is used for this non-thermal decomposition technique (Figure 11.28). Gamma radiolysis is effective in PCDD degradation and can also be used in the disinfection of wastewaters. A surfactant is added to mobilize TCDD molecules to a more appropriate site in the soil. The addition of promoters (active carbon, $Cu_2O$) to the toxicants improves the degradation percentage under electron beam radiation.

A concept of direct and indirect interaction of γ radiation with dioxin (TCDD) contaminated soil particles is illustrated in Figure 11.28. The direct effect of the γ-ray result from the ionization of the target compound (electrons are emitted) is depicted along path a. Along path b, a thermalized electron emitted by an adjacent ionized molecule interacts with the target compound to cause a chemical transformation. In this case, reductive dechlorination may result in the presence of a hydrogen donor; for instance, a natural organic material (NOM) in the case of soil. The indirect effects of the γ-ray occur from the radiolysis of the solvent, due to which radicals are formed, which diffuse to the target compound resulting in chemical transformation (Source: Hilarides and Gray, 1994).

*Advantage:* More than 92% efficiency of radiolytic degradation for 2,3,7,8-TCDD removal is reported (Gray and Hilarides, 1995).

*Disadvantage:* Major disadvantage is unidentified.

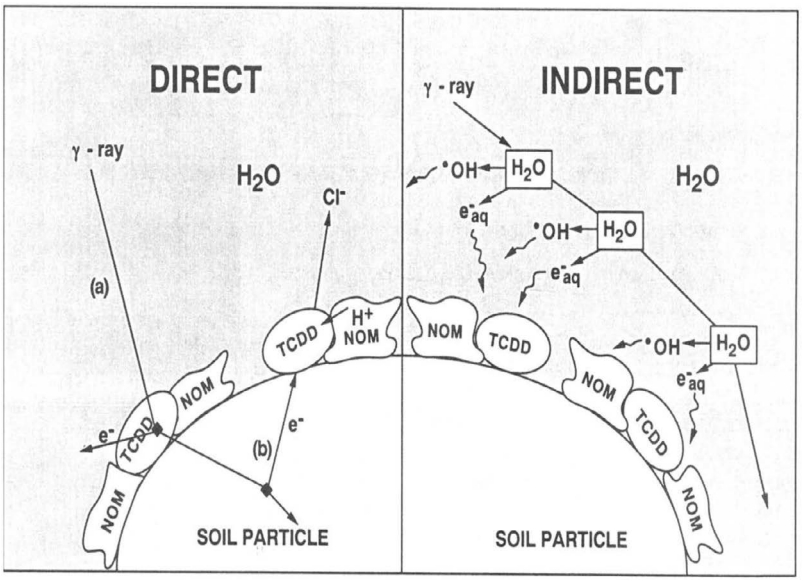


*Figure 11.28* Conceptual diagram of direct and indirect interaction of γ radiation with dioxin (TCDD) contaminated soil particles.

### 4.2.15 Base catalyzed decomposition (BCD) method (Dechlorination methods)

*Principle mechanism:* A chemical dehalogenation process in which an alkali or alkaline earth metal carbonate, bicarbonate, or hydroxide is added to the polluted medium in a moderate temperature thermal desorber (MTTD) at temperatures extending between 315–426 ° C. Alkali ($NH_3$) is supplemented to the contaminated medium, followed by a hydrogen donor compound (Na/Ca) if the contaminated material lacks hydrogen ions (Chen *et al*., 1997). The BCD method (Figure 11.29) chemically detoxifies the chlorinated organic pollutants by replacing chlorine with hydrogen.

*Advantage:* BCD is an environment-friendly and economical method of fly ash detoxification. The concentration of each isomer of PCDDs and PCDFs were found to be significantly reduced by this method.

*Disadvantage:* No significant disadvantage is reported.

### 4.2.16 Subcritical water (SCW) treatment

*Principle mechanism:* Water held in the liquid state above 100 °C by applying pressure is termed as subcritical water, which is used to extract dioxins from soil and sediments. Application of zero-valent (ZVI) iron in reductive dechlorination of PCDDs and treatment of polluted soils with subcritical water as the reaction medium and the extractive solvent is found to be effective (Kluyev *et al*., 2002). A schematic diagram of the SCW apparatus is shown in Figure 11.30.

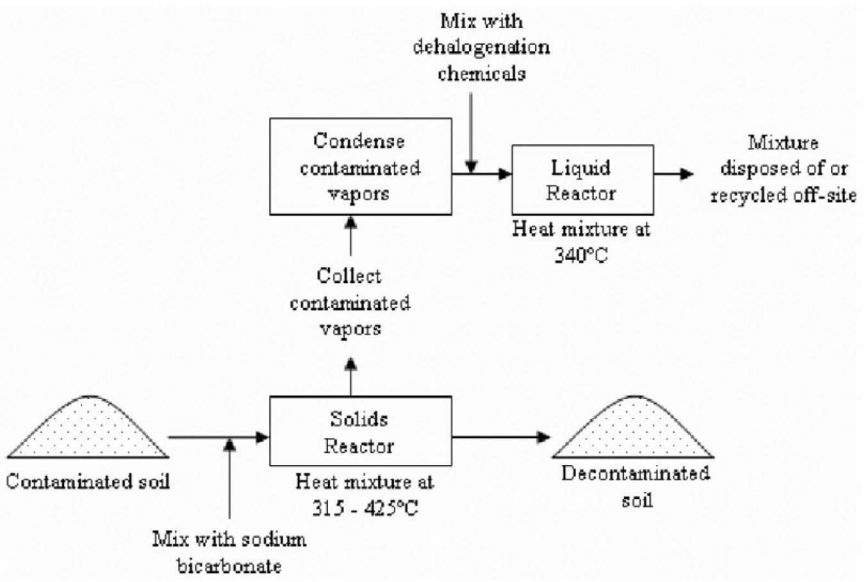


*Figure 11.29* Base catalyzed decomposition (BCD) method (dechlorination methods).

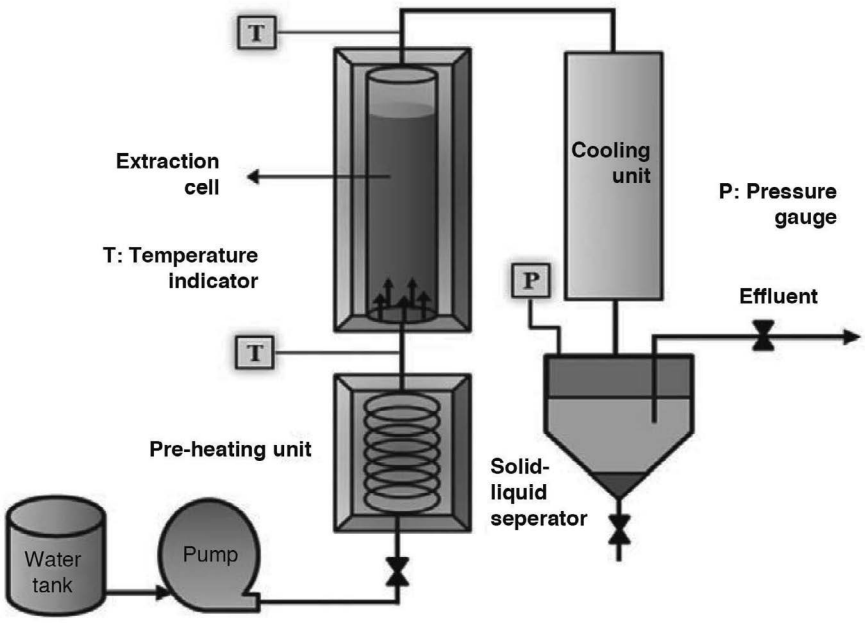


*Figure 11.30* Schematic diagram of the lab-scale subcritical water oxidation (SCW) treatment apparatus.

(Source: Islam *et al.*, 2015)

*Advantage:*Hashimoto *et al*. (2004) achieved 99.4% dioxin removal by this treatment method at 350 °C within 30 min. The zero-valent (ZVI) iron used is inexpensive, easy to handle, and effective in remediating a wide variety of chlorinated compounds. Subcritical water treatment is widely applied as in-situ, ex-situ, or as part of a controlled remediation procedure in wastewater, drinking water, mine tailing applications, soil amendment, and stabilization (Kluyev *et al*., 2002).

*Disadvantage:* No disadvantage is reported.

### 4.2.17 Liquefied gas solvent extraction (LG-SX) technology (for soil and wastewater)

*Principle mechanism:* The liquefied gas solvent extraction process (Figure 11.31) involves physico-chemical methods of separating organic pollutants from soil and sediment (Kulkarni *et al*., 2008). It uses liquefied gas solvents, such as liquefied carbon dioxide and propane, to extract organics from soil (Saldana *et al*., 2005). The low densities, viscosities, and surface tensions of these gases result in substantial extraction in comparison to conventional liquid solvents. Contaminated solids, slurries, or wastewaters are fed into the extraction system along with the solvent, and after the separation of solvent and organics, the mixture passes to the solvent recovery system. The organics are either reused or disposed of in soil and sediments.

*Advantage:* This method produces clean soil and sediment that can be used in the restoration of the site (Silva *et al*., 2005). Liquefied gas has higher rates of extraction than conventional liquid solvents. As a result of high volatility, gases can be easily recovered

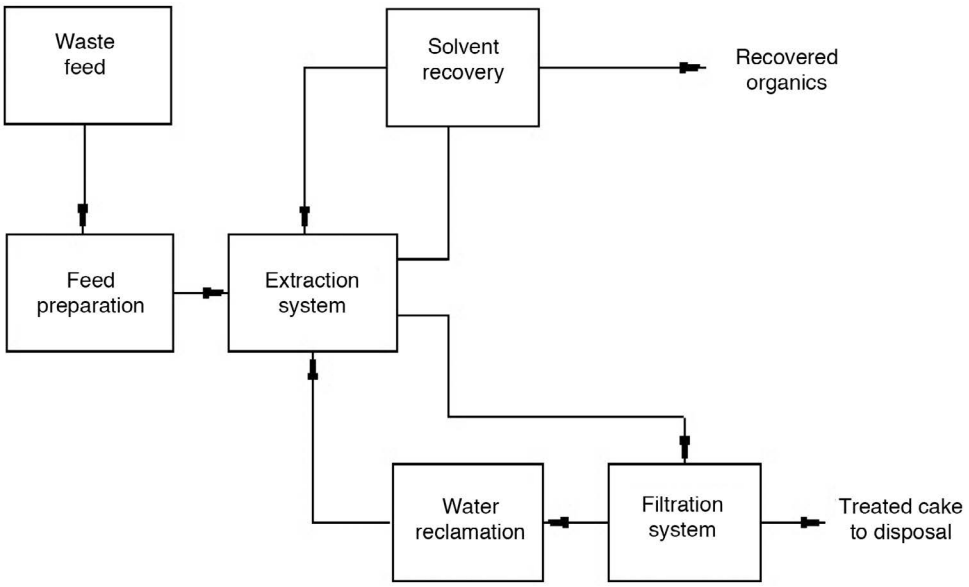


*Figure 11.31* Flow diagram of liquefied gas solvent extraction (LG-SX) technology.
(Source: https://clu-in.org/products/site/complete/democomp/cfsys.htm)

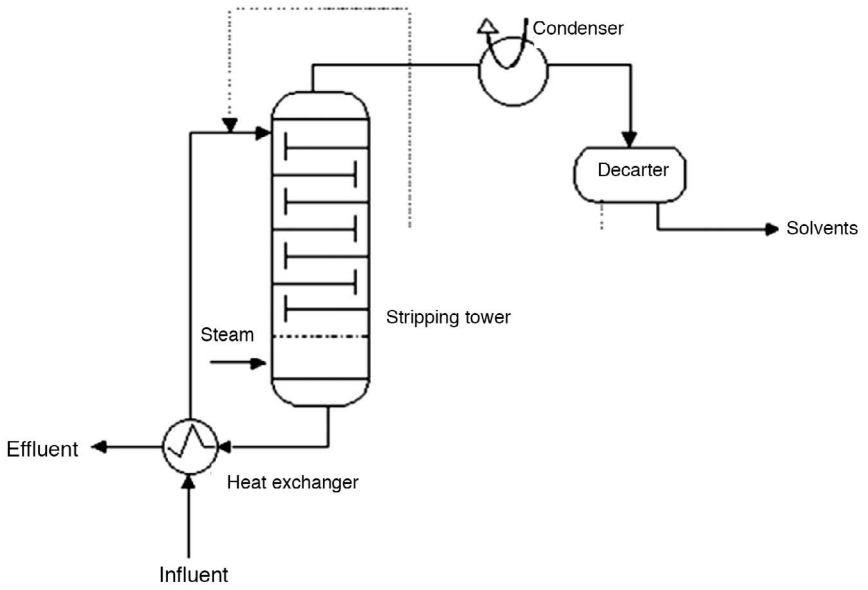


*Figure 11.32* Typical steam distillation process.
(Source: https://emis.vito.be/en/techniekfiche/steam-stripping)

from the suspended solids matrix and this minimizes solvent losses. Ninety-nine percent of the organics are extracted from the feed, and the solvent used is recycled for further use.

*Disadvantage:* This is an ex-situ procedure which needs excavation of the contaminated soil.

### 4.2.18 Steam distillation (soil/sediments/fly ash)

*Principle mechanism:* The steam distillation process (Figure 11.32) involves vaporization of the volatile components of a liquid mixture. It takes place by providing open steam at a lower temperature, i.e. below the boiling point of either of the pure liquids (Kulkarni *et al*., 2008). Efficiency increases when used with microwave energy. In the microwave method (Figure 11.33), heat is produced within the material, rather than instigating from external sources to remove volatile and semi-volatile organic contaminants from the soil without degradation.

*Advantage:* Steam distillation is a simple and economical method of dioxin removal.

*Disadvantage:* Microwave energy is required for high efficiency.

### 4.2.19 Mechanochemical (MC) technology

*Principle mechanism:* In the mechanochemical technique (Figure 11.34), the mechanical energy is carried to the solid system from the milling bodies by shear stresses or compression (Kulkarni *et al*., 2008). A considerable part of the milling energy is transformed into heat, and a little energy is used to apply breaks, stretches, and compression at the micro and

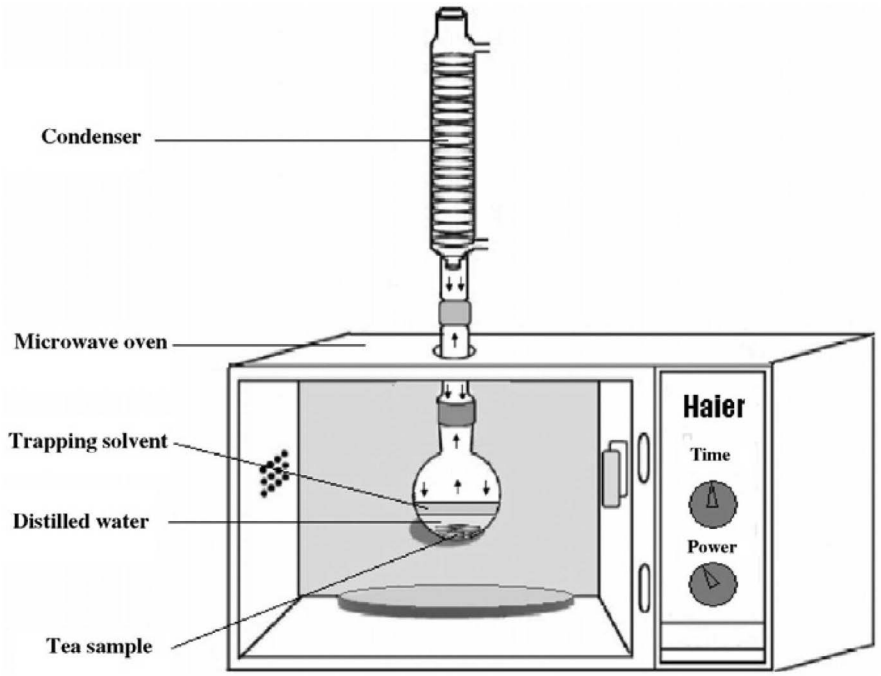


*Figure 11.33* Extraction apparatus of the microwave-assisted steam distillation process.
(Source: Ji *et al.*, 2007)

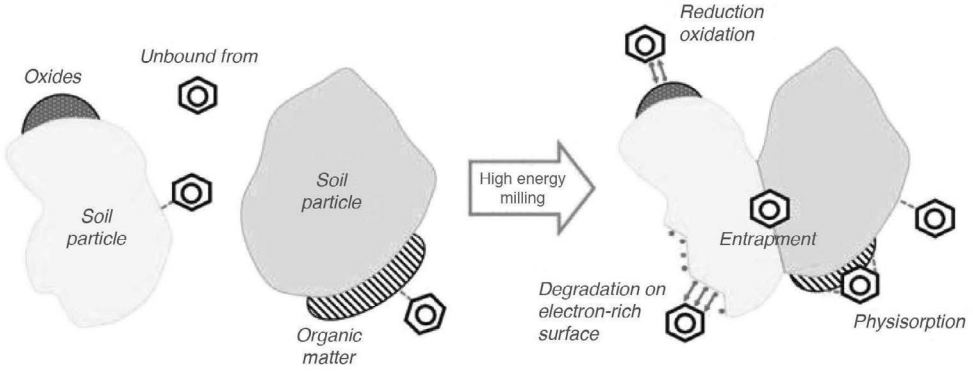


*Figure 11.34* Mechanochemical phenomena occurring during organic pollutant degradation and destruction by ball milling.
(Source: Cagnetta *et al.*, 2018)

macroscopic level or to complete the reaction. This process requires a dechlorinating reagent, for instance, Al/CaO (Mio *et al*., 2002; Napola *et al*., 2006).

*Advantage:* Mechanochemical technology is a novel ex-situ method for dioxin treatment. This is an efficient method for the complex structure and sturdy nature of the contaminant. Owing to the use of a portable facility, it is possible to dispose of organic wastes at any desired sites. The method has many economic and ecological benefits viz. requirement of low energy input, no harmful emissions, and noxious compounds can be transformed into defined and useful products.

*Disadvantage:* No disadvantage is reported for MC technology.

## 5 Conclusion

Dioxin compounds are biologically persistent environmental toxicants due to which there is a very high health risk to a human being. Dioxins can damage the immune system, reproductive system, endocrine system, and can cause all types of cancer. Major sources of dioxins in the environment are incineration and other combustion processes, and thus, a greater understanding of the precursor and de novo mechanisms of dioxin formation are required. The interaction between chlorine and precursors must be well construed.

Furthermore, the conditions are important under which carbon, oxygen, and chlorine can turn out to be limiting reactants in the incineration and combustion chamber. The relationship between the rates of carbon consumption and dioxin formation should be better explicated. Exposure to dioxins can be minimized by adopting some strategies, i.e. by a balanced diet that is low in fats and by replacing polyvinyl chloride (PVC) with chlorine-free materials. Dioxin formation in flue gas can be avoided by manipulation of the process parameters, for instance, residence time, temperature, the turbulence of the combustion chamber, and post combustion flue gas treatment facilities, etc. Numerous technologies are also available for dioxin remediation or removal from gases. Waste incineration plants generally use baghouse filters (fabric filters) equipped with activated carbon injection, or fixed bed carbon filters to abate dioxin emission. SCR-catalysts (selective catalytic reduction) for NOx reduction in conjunction with an oxidation catalyst is also an effective method to destroy dioxins. The other novel technologies involve the installation of systems of catalytic destruction of dioxins and the use of efficient filter materials. Ceramic filters can be used for the continuous removal of particles from air or other gases. Such materials are also resistant to chemical corrosion and withstand high temperature (>450 °C). In the year 2000, W.L. Gore and Associates developed a system to destroy dioxins by the catalytic filters REMEDIA D/F, which consist of membranes of expanded PTFE, containing the catalytic system (Gore, USA). In this method, the fine particles are trapped on the membrane or the surface of the filter but allow the PCDD/Fs to pass through the membrane, which instantaneously reacts with the catalyst giving $CO_2$, $H_2O$, and HCl as products. The decontamination of soil, sediments, and fly ash from dioxins is a wide area of research. The current practice of ex-situ thermal treatment processes is energy consuming, and hence, alternative techniques are needed to check the energy consumption. The reuse of treated soil is strongly limited due to the loss of organic matter at high temperature. In ex-situ methods, the transfer of dioxins from the source to the treatment site is a complicated process. Diverse sources introduce heterogeneous and complex mixtures of dioxins into the environment; no single congener can be used to

attribute the dioxin occurrence in a sample to a specific source. Thus, enhanced transport mechanisms are to be developed, the photolytic techniques seem to be very cost-effective for in-situ remediation of dioxins, but their photodegradation efficiency mainly depends on the sunlight availability. The use of supercritical water for the treatment of dioxins present in the soil and fly ash looks to be a promising one. However, more investigations are needed in order to make the process realistic. Steam distillation, solvent, liquefied gas extraction, and mechanochemical are forthcoming technologies and may have the potential to efficiently remove dioxins from contaminated soils. However, a single pilot-scale investigation (USEPA) on the application of liquefied gas for PCB removal from soil exists and the current information is very limited on the removal of toxic, high molecular weight dioxin compounds. Biodegradation is the most economical amongst all the methods described for the remediation of dioxins; however, the development of efficient hybrid organisms in the laboratory is required for the maximum decomposition of these compounds. Since biodegradation is a time-consuming process, due to the low bioavailability of dioxins, their rates can be enhanced by the application of bio-emulsifiers and chemical pre-treatment of the soil and sediments. In our opinion, the application of liquefied gas, supercritical water, and biodegradation (along with the chemical pre-treatment) have greater potential. Still, there is a need for more investigation for the development of sustainable remediation methods.

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Chapter 12

# Environmental risks and bioremediation of dioxins

*Mandeep Dadhwal, Poonam Sharma and Indrakant Kumar Singh*

## 1 Introduction

Dioxins are a set of chlorinated organic compounds having identical chemical structure, commonly classified as polychlorinated dibenzo-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs). On the benzene ring, numbered from 1 to 8, chlorine atoms can be present at eight different positions. The toxicity and harmful effects of dioxins depend on the amount and positions of the chlorine atoms. If one chlorine atom is present on dibenzo dioxins, the compound is known as mono-dibenzo dioxin. The most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), carries four chlorine atoms (Figure 12.1). The biochemical properties of dioxin are more like polychlorinated benzene due to the presence of benzene rings and chlorine atoms. However, there are numerous applications of polychlorinated benzene in industry but no known application of dioxins. Dioxins are insoluble in water and have strong affinity towards lipids. Dioxins are mostly produced by anthropogenic activities but are also naturally produced. Incomplete combustion of organic compounds by fires and volcanic activity leads to their natural production. Various industrial, municipal, and household incineration and combustion activities inadvertently lead to their production. Presently, the main processes of dioxin production in the environment are human incineration and combustion activities (WHO, 2016). Dioxin production also occurs in the pulp and paper industries and during the manufacturing of certain organochlorine compounds such as chlorinated phenol. During the production of 2,4,5-trichlorophenol (2,4,5-TCP), a by-product 2,3,7,8-TCDD is produced. 2,4,5-TCP is used in the production of a disinfectant, hexachlorophene, and a herbicide, 2,4,5- trichlorophenoxyacetic acid (2,4,5-T). Dioxins have also been found in cigarette smoke, heaters, and exhaust from cars. Combustion of several organic compounds that may contain chlorine and carbon, such as plastics, paints, wood treated with pentachlorophenol (PCP), pesticides, and polychlorinated chemicals also produce dioxins. The objective of this chapter is to describe dioxin toxicity, persistent nature, dioxin degrading microorganisms, and the further scope of these microorganisms in dioxin remediation from the environment.

## 2 Dioxins are persistent in nature

Dioxins are resistant to degradation, they are carried far away from the places of release via air, water, and organisms (like migratory organisms), and finally settle and accumulate in both terrestrial and aquatic ecosystems. Owing to their ubiquitous and persistent nature,

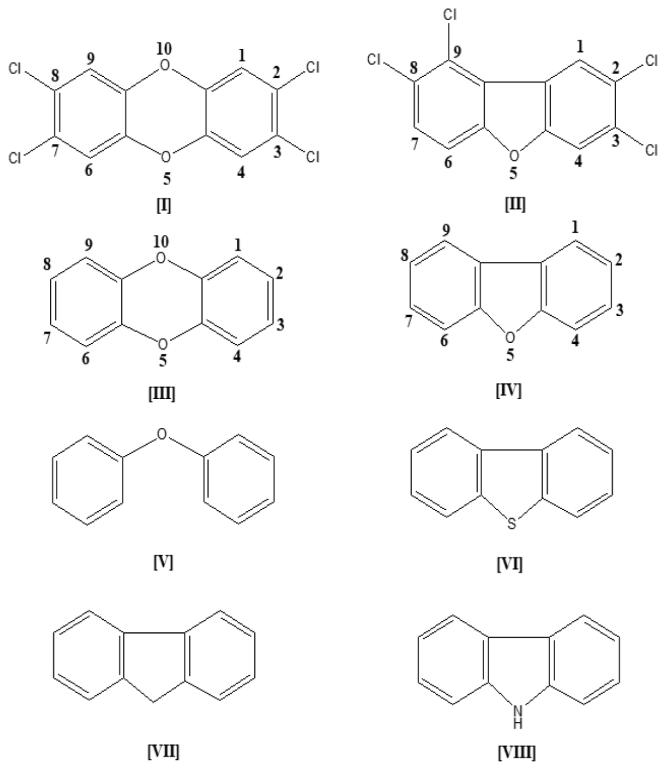


*Figure 12.1* Chemical structure of dioxins; I. 2,3,7,8-Tetrachlorodibenzo para dioxin (TCDD), II. 2,3,7,8-Tetrachlorodibenzofuran (TCDF), III. Dibenzo-p-dioxin (DD), IV. Dibenzofuran (DF), V. Diphenyl ether (DE), VI. Dibenzothiophene (DBT), VII. Fluorene (FN), VIII. Carbazole (CAR).

(Source: Hiraishi, 2003

dioxins bioaccumulate in food chain(s). Hence, the majority of the human population is contaminated with PCDD/Fs and PCBs (WHO, 2016). Furthermore, since dioxins bioaccumulate in humans and pass from mother to fetus via placenta and from mother milk to babies (WHO, 2008), infants are at higher risk in the early stage of their growth (Nishijo *et al*., 2012)

Since dioxins are fat soluble, they easily accumulate in humans and animal fat tissues. Foods derived from animals are the main cause of dioxin exposure to humans. Due to the lipophilic nature of dioxins, they are found in higher concentration in dairy products, poultry, eggs, meat, and fish (Eduljee and Gair, 1996), and lower concentration in green vegetables, fruits, and grains. The toxicity of the dioxin mixture is measured in the form of toxic equivalent (TEQ). The TEQ scheme weighs the toxicity of less toxic compounds as fractions of the toxicity of the most toxic 2,3,7,8-TCDD component of dioxin mixture. Each congener has specific "Toxic Equivalency Factor" (TEF). TEF is calculated on the basis of the toxicity data of various dioxin congeners. TEF indicates the toxicity of each congener as compared to 2,3,7,8-TCDD, which is given the reference value 1. All other

dioxin congeners have TEF values less than one. To calculate the total toxic equivalent (TEQ) of a dioxin mixture, the amount (weight) of each dioxin congener is multiplied with their TEF and then added together. Schecter *et al*. (1997) measured dioxin concentrations in different food samples that were collected in 1995 in supermarkets across the U.S. Freshwater fish had the highest level of dioxins (1.43 TEQ1), followed by butter (1.07 TEQ), hotdog/bologna (0.54 TEQ), ocean fish (0.47 TEQ), cheese (0.40 TEQ), beef (0.38 TEQ), eggs (0.34 TEQ), ice cream (0.33 TEQ), chicken (0.32 TEQ), pork (0.32 TEQ), milk (0.12 TEQ), and vegetables, fruits, grains, and legumes (0.07 TEQ). Thus, the concentration level of dioxin contamination in an individual's diet is based on the type of food and its consumption. Patandin *et al*. (1999) conducted a survey regarding dioxin intake through different food items among a group of nursery school children in the Netherlands and found that dairy and milk products were the main contributors (50%) towards intake of dioxins and similar compounds through food, while meat and processed foods were only 20% and 25% contributors, respectively.

## 3 Toxicity of dioxins and related compounds

PCDDs and PCDFs toxicity in many animal species has been experimentally studied; however, toxicology studies are mainly done with 2,3,7,8-TCDD. The toxicity (LD50) differs significantly with species, strain, age, and also route of administration. Therefore, LD50 (μg/kg) for oral administration varies between guinea pigs and Syrian hamsters. The most common toxic effect of dioxins includes increased weight loss, less appetite, thymus atrophy, and gastrointestinal hemorrhage. Furthermore, dioxin also affects liver, skin, and endocrine glands (Fact Sheet, 2008). No confirmation has been obtained to validate the TCDD-induced formation of addition product(s) with proteins or nucleic acids (Poland and Glover, 1979) or cellular DNA damage (Shu *et al*., 1987). Nevertheless, AHR (aryl hydrocarbon receptor) protein has been reported to mediate numerous toxic effects of TCDD. The receptor mediated mechanisms involve a series of organized steps: (a) Dioxin entry into the cells, (b) AHR and binding of dioxins, (c) receptor-ligand complex attachment on regulatory sites of gene, (d) activation and translation of protein kinase and phosphatase producing genes. and (e) finally. the mode of action of the expressed proteins (Landers and Bunce, 1991)

One of the several unanswered questions in toxicity of dioxin is why organisms have a receptor for TCDD at all? Dioxins and related compounds are produced in the environment in high concentrations due to anthropogenic activities; therefore, development of such receptor is highly unbelievable. One theory suggests that the AHR evolved to detoxify the products of combustion (fire) such as benzopyrene, which like TCDD, binds to the AHR with high affinity and triggers the production of several proteins/enzymes. This suggests that the binding of TCDD to the AHR is actually accidental due to broader substrate specificity (Landers and Bunce, 1991). Jakoby and Ziegler (1990) have reported that the enzymes responsible for detoxification of xenobiotic compounds have broader specificity unlike the familiar hydrolytic and oxidative enzymes, which are characterized by pronounced substrate specificity. These enzymes are promiscuous in nature to catalyze incorporation of different functional group.

Numerous studies have been carried out to understand how dioxins cause cancer or are related to the development of cancer in humans. The most revealing epidemiologic studies

are those that were done on the human population of Seveso, who accidentally got exposed to dioxins (TCDD) in 1976, and the workers were exposed to a very high concentration of dioxin and other chemicals in a manufacturing plant. The various studies have been done to study the effect of dioxins on general population. The exposure levels were 1,000 times higher in the exposed population as compared to the unexposed population (Bertazzi *et al*., 1998)

The result published on risk of cancer due to the effect of dioxin indicates the following observations (INSERM, 2000). First, dioxin-related compounds act as carcinogens that affect the risk of cancer but there is no correlation between dioxins and a specific cancer. Second, the high risk of cancer found in worker was not by chance since the results were statistically very significant. In spite of this, these results must be examined carefully, in view of the smallness of the global risks. Effects of other factors, such as smoking or exposure to industrial chemicals, cannot be totally ruled out. Finally, the dioxin exposure levels of the study of subjects was very high as compared to the general population. Therefore, the applicability of these results for general populations would require further studies to determine if the effects at low doses are the same as high doses. In the current scenario, not a single case of cancer is clearly attributed to only dioxin exposure in the general population. Other toxic effects like dermatological effects, neurological disorder, change in thyroid function, and cardiovascular effects have been reported in the dioxin-exposed population of Seveso, but these results are not consistent and are based on few observations.

The immunotoxic effects of dioxins on humans are still not clear. The studies done in this field are not complete; the type of agent (dioxin or dioxin-like), concentration of the agents, and the exposure duration are not mentioned clearly in these studies. Hence, it is very difficult to ascertain the immunotoxic potential of dioxins and related compounds with these extremely variable results.

## 4 Degradation of dioxins

Dioxin decontamination from the environment is one of the most challenging problems due to their toxic and persistent nature. Physicochemical techniques for decontamination of dioxins like thermal remediation, photodegradation, supercritical hydrolysis using water, and dechlorination with a metal catalyst have been developed and used. However, application of physicochemical methods for large dioxin-contaminated fields is not feasible. Microorganisms play a crucial role in the degradation and mineralization of xenobiotics and toxic compounds in the ecosystem. They are present at the base of the food chain and are the first organisms to interact with xenobiotic compounds. Hence, microbial degradation of dioxins has greater potential than physicochemical techniques in their application for environmental remediation. The large number of studies already done on the microbial degradation of dioxins, and the various microorganisms capable of degrading dioxins and related compounds have been isolated and studied. Dioxin degradation pathways, as well as the biodiversity and biochemical properties of dioxin-degrading microorganisms, have been very well studied in past few years, but bioremediation techniques for dioxin-polluted sites are yet to be developed (Armengaud and Timmis, 1997; Bressler and Fedorak, 2000; Nojiri *et al*., 2001; Nojiri and Omori, 2002; Wittich, 1998). The aromatic hydrocarbon dioxygenases are involved in the degradation of the

ring structure of dioxins in aerobic bacteria. In addition to bacteria, fungi are also involved in the degradation of dioxins. The fungal degradation of dioxins occurs with the enzyme system cytochrome P450, lignin peroxidase and laccase (Hammel *et al*., 1986; Joshi and Gold, 1994).

## 5 Aerobic degradation of dioxins

The oxidative degradation of dioxins is done by aerobic bacteria (Bertini *et al*., 1995; Engesser *et al*., 1989; Erickson *et al*., 1992; Fukuda *et al*., 2002; Gieg *et al*., 1996; Hong *et al*., 2000; Takase *et al*., 1986). The first enzyme involved is aromatic ring dioxygenase, which has been a very well-studied enzyme. There are two dioxin oxidation pathways, one is termed lateral deoxygenation in which the aromatic ring is attacked at the lateral 1,2 position or rarely 2,3 or 3,4 positions and transformed to cis-dihydrodiols. The second pathway is angular deoxygenation, which takes place at the angular position 4 and adjacent to the ether bridge. A large number of dioxin degrading bacteria are from the family Sphingomonadaceae (Table 12.1). Although the exact reason is still not clear, it is postulated that the sphingolipids present in the cell membrane of these bacteria carry high affinity to dioxin-like compounds (Hiraishi, 2003)

*Table 12.1* Bacterial strains capable of degrading dioxins and dioxin-related compounds via deoxygenation.

| *Bacteria* | *Substrate degraded* | *Mode of dioxygenation* |
|---|---|---|
| Class ***Alphaproteobacteria*** | | |
| *Porphyrobacter sanguineus* IAM 12620$^T$ | DF | Lateral |
| *Sphingobium yanoikuyae* B1 (=DSM 6900) | DD, DF | Lateral |
| *Sphingomonas* sp. HH19k | DF | Lateral |
| *Sphingomonas* sp. HH69 (=DSM 7135) | DD, DF | Angular |
| *Sphingomonas* sp. HL7 | DF | NA |
| *Sphingomonas* sp. RW16 (=DSM 12667) | DF | Angular |
| **Class Betaproteobacteria** | | |
| *Burkholderia cepacia* F297 | DF, DBT, FN | Lateral |
| *Burkholderia* sp. JB1 | MCDD/F | Angular |
| *Burkholderia* sp. LB400 | DD, DF | Angular |
| *Ralstonia* (*Wautersia*) sp. DSM 6708 | MCDD/Fs,DCDD/Fs | NA |
| *Ralstonia* (*Wautersia*) sp. SBUG290 | DF, BP | Lateral |
| **Class *Gammaproteobacteria*** | | |
| *Erwinia* sp. CU3614 | DE,MCDD,DCDD | NA |
| *Klebsiella* sp. HL1 | DF | NA |
| *Pseudomonas fluorescens* BS243 | DF | Lateral |
| *Pseudomonas putida* BS291 | DF | Lateral |
| *Pseudomonas* sp. NSS2 | Carboxy DE | Angular |
| *Pseudomonas stutzeri* OM1 | CAR | Angular |
| **Class *Actinobacteria*** | | |
| *Arthrobacter* sp. F101 | FN | Lateral |
| *Brevibacterium* sp. DO | DBT,FN | Angular |
| *Brevibacterium* sp. DPO220 | DF,FN | Angular |

(Source: Hiraishi, 2003)

## 6 Biodegradation via lateral deoxygenation

In the lateral pathway, the dioxygenase enzyme acts on lateral 1,2 and 2,3 positions of one of the aromatic rings with high regioselectivity (Figure 12.2). The dihydrodiols produced in the dibensofuron (DF) degradation pathway are converted to the dihydroxy compound, and then *meta*-cleavage of dihydroxy-DF occurs. Due to this cleavage of the aromatic ring, a

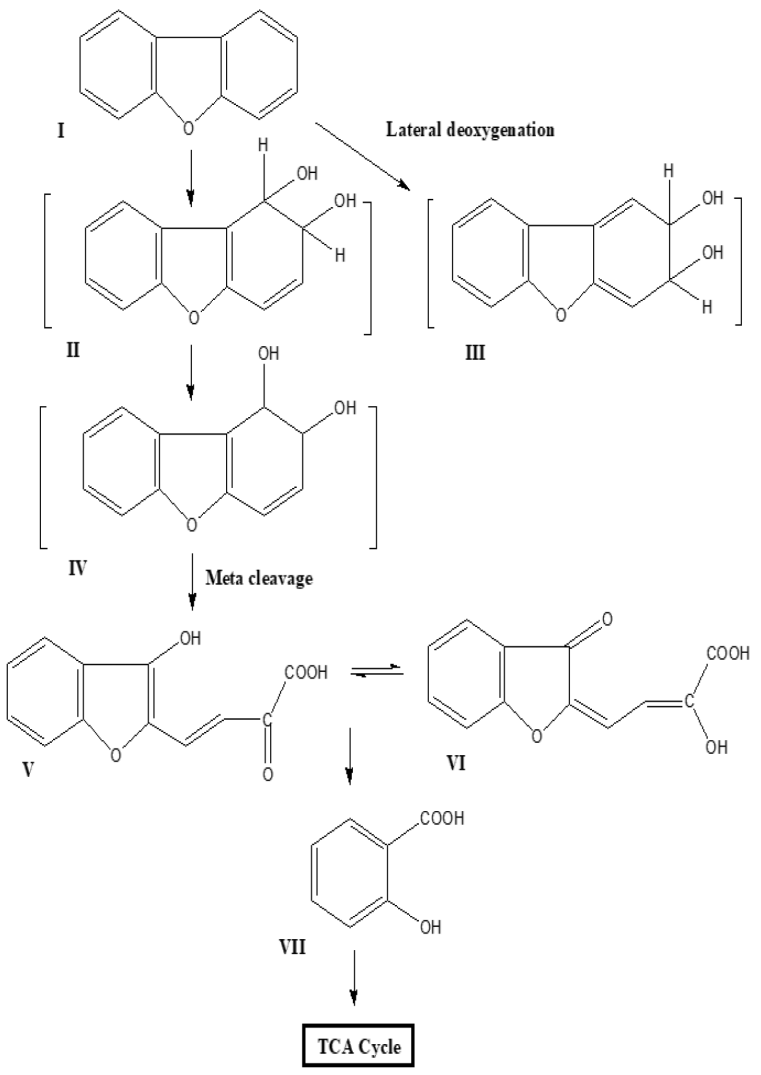


*Figure 12.2* Lateral deoxygenation pathway of dibenzofuran (DF). The following intermediates formation occurs from I. DF; II. 1,2-dihydro-dihydroxydibenzofuran; III. 2,3-dihydro-dihydroxydibenzofuran; IV. 1,2-dihydroxydibenzofuran; V. 2-oxo-4-(3'hydroxybenzo-furan-2-yl)-but-3-enoic acid; VI. 2-hydroxy-4-(-3'-oxo-3'H-benzofuran-2'-yliden)-but-2-enoic acid; VII. Salicylic Acid.

(Source: Hiraishi, 2003)

polar yellow intermediate that has an absorption maximum at around 460 nm at pH 7.0 is produced. In some bacteria, degradation of DF and its relatives via lateral dioxygenation is a co-metabolizing process during growth with other organic compounds (naphthalene or biphenyl) as the carbon and energy source. The lateral deoxygenation leads to a dead end product; therefore, these bacteria are unable to degrade DF completely (Wittich *et al*., 1998). Recently, the aerobic photosynthetic bacterium, *Porphyrobacter sanguineus* strain IAM 12620 as well as *Sphingobium yanoikuyae* strain DSM 6900 (strain B1) are reported to be capable of growing with DF as the sole carbon source, giving the yellow intermediate metabolite (Hiraishi *et al*., 2002).

## 7 Biodegradation via angular deoxygenation

In the angular deoxygenated pathway, an initial dioxygenase attack occurs at the angular positions on the two carbon atoms adjacent to the ether bridge (Figure 12.3) with high specificity (Engesser *et al*., 1989), which leads to the formation of the corresponding *cis*-dihydrodiols. The hemi-acetal products are spontaneously transformed to 2,2',3-trihydroxydiphenyl ether (Figure 12.3) and 2,2',3-trihydroxybiphenyl, respectively. The dihydroxylated rings of the products are then *meta*-cleaved and lead to the formation of catechol and salycilic acid after hydrolysis of the *meta*-cleavage products in the DD and DF metabolic pathways. The angular deoxygenation pathway has been found in several species of the phyla *Actinobacteria* and *Proteobacteria* (Table 12.1). *Sphingomonas wittichii* strain RW1 is one of the most intensively studied organisms in this respect and the metabolic products for DD and DF in this bacterium have been identified (Engesser *et al*., 1989).

## 8 Biodegradation of chlorinated congeners

Chlorine substituted dioxins are degraded via the initial deoxygenation pathway. Despite its ability to degrade dichlorinated and higher chlorinated DDs and DFs, *Sphingomonas wittichii* strain RW1 is unable to grow with almost any of these congeners as sole carbon sources. Some of the metabolites produced during the degradation process are resistant to further degradation. However, the complete degradation of 4-MCDF was found in a co-culture of strain RW1 and bacterium, *Burkholderia* sp. strain JWS (Arfmann *et al*., 1997).

In this case, a dioxygenolytic attack by strain RW1 took place at the non-chlorinated aromatic ring, as the other position is sterically hindered. This degradative reaction resulted in the production of the dead-end product 3-chlorosalicylate. This product was then further degraded by strain JWS. The rate of dioxin degradation depends upon the presence of chlorine atoms around aromatic rings and decrease with increasing chlorine atoms around the aromatic rings. In particular, dioxygenolytic attack on the diaryl ether linkage is difficult, if chlorine atoms are present at positions 1, 4, 6, or 9. Therefore, the possibility of complete dioxin degradation by single dioxygenase-producing aerobic bacteria is limited to TCDD/Fs and lesser chlorinated dioxins.

## 9 Fungal degradation

Many species of fungi possess enzymes which attack lignin and other organic polymers and can also use such a multienzyme system to degrade aromatic compounds like dioxins.

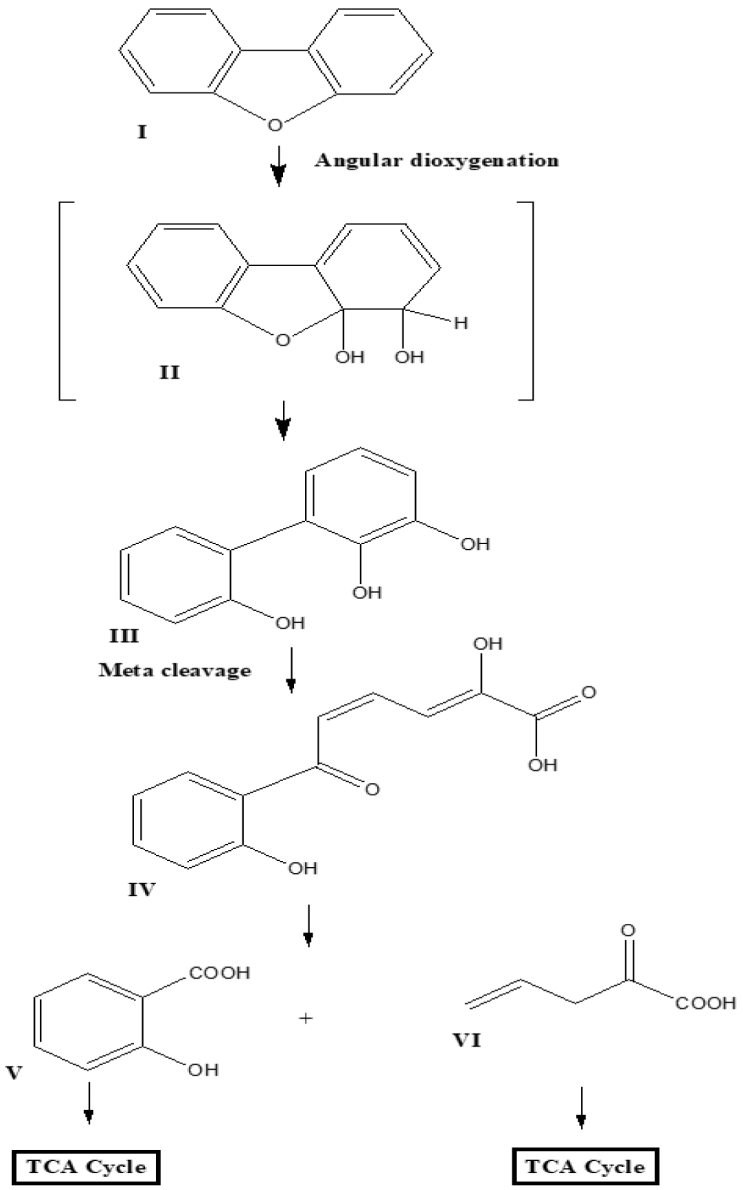


*Figure 12.3* Angular deoxygenation pathway of dibenzofuran I. DF; II. 4, 4a-dihydro-dihydroxy dibenzofuran; III. 2, 2', 3-trihydroxybiphenyl; IV. 2-hydroxy-6-(2-hydroxy phenyl)-6-oxo-2, 4-hexadienoic acid; V. Salicylic Acid; VI. 2-oxo-4-pentenoate. Compounds in square are not detected.

(Source: Hiraishi, 2003)

Enzymes involved in the degradation of aromatic compounds are cytochrome P450 (P450) and extracellular lignolytic enzymes, lignin peroxidase (ligninase), and laccase. P450 is an iron protein which is widely distributed in eukaryotic organisms as well as in prokaryotes. The P450 system is involved not only in hydroxylation of organic compounds in biosynthesis pathways but also in detoxification of xenobiotic compounds. The P450 protein is responsible for the multiple hydroxylation of DF that is widely present among fungi. Hydroxylation or utilization as a carbon and energy source of DF, DE, and related aromatics has been reported in *Cunninghamella elegans* (Cerniglia *et al*., 1979), *Cunninghamella echinulate* (Seigle-Murandi *et al*., 1991), and *Penicillium canescens* (Hammer *et al*., 2001), although these reports have not always demonstrated the involvement of P450. The proposed pathway for the early steps of DF degradation by *Trichosporon mucoides* shows the formation of 2,3-hydroxy DF followed by the ring cleavage, leading to the formation of 2-(1-carboxy methylidene)-2,3-dihydrobenzo[*b*]-furanylidene glycolic acid (Hammer *et al*., 2001). The degradation mechanism of dioxin is different in fungi as compared to bacteria.

## 10 Bioremediation of dioxin-contaminated soil

The laboratory scale biodegradation studies showed that the concentration of 2,3,7,8-TCDD incorporated into the soil was reduced to some extent due to microbial activity. Indigenous microorganisms in polluted soils have the ability to degrade dioxins. The rate of degradation can be slow or fast depending on the type of microorganism and various environmental factors. Rosenbrock *et al*., 1997 studied the potential for biodegradation of 14C-labeled TCDD in soil. An increase in the carbon content by the addition of organic compounds enhanced DD mineralization by soil microorganisms. A white rot fungus, *Ceriporia* sp. strain MZ-340 was used for the bioremediation of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans contaminated fly ash (Suhara *et al*., 2003). Halden *et al*., 1999 studied the degradation of DD, DF, and 2-monochlorodibenzo-p-dioxin (2-MCDD) from soil microcosms by strain RW1. In this case, the degradation rate of 2-MCDD depended upon the concentration of the cell added and the content of soil organic matter. Another lab scale experiment with *Sphingomonas* sp. strain KA1 showed 96% and 70% degradation of 2-MCDD and 2,3 dichlorodibenzo-p-dioxin (2,3-DCDD), respectively, after seven days of incubation (Habe *et al*., 2002).

There are also some reports available on model bioremediation processes using a particular bacterium other than the sphingomonads. In a soil microcosm study containing chlorinated DDs and DFs, a single inoculation with *Pseudomonas resinovorans* strain CA10 at cell densities of 107 and 109 CFUg – 1 soil resulted in the degradation of 46% and 80% of 2,3-DCDD, respectively, during seven days of incubation (Habe *et al*., 2002). Hiraishi *et al*., 2002 reported bioremediation of dioxin-polluted soil via biostimulation. When highly contaminated soil was incubated with sterile compost as an organic nutrient additive, the indigenous microorganisms in the soil decreased concentrations of PCDD/Fs by 22% after three months. In this case, the indigenous microbial populations in the dioxin-contaminated soil have a potential for PCDD/Fs degradation. Also, the addition of compost-enhanced microbial activity in soil, consequently, increased the rate of PCDD/Fs degradation. These reports indicate the possibility of decreasing PCDD/F congeners actually present at polluted sites by a single inoculation with a particular organism. However, all these bioremediation

studies using a particular organism are preliminary, usually done only on laboratory scale, and it is clearly necessary to study this on a large field scale. Furthermore, studies are required to ascertain if bioaugumentation with a single aerobic bacterium is actually effective for *in situ* bioremediation.

As reported in the various studies, the bacterial dioxygenases specificity is limited to one or di-chlorinated congeners like MCDD/Fs. The complete degradation of higher congeners like 2,3,7,8-TCDD and other toxic PCDD/Fs by using only an indigenous bacterium is actually difficult to achieve. There is a need to explore the microbial community/enzyme systems which can degrade these higher congeners effectively. Bacterial consortium is another alternative for effective degradation of higher chlorinated congeners. The lignin degradative enzymes of white-rot fungi or Basidiomycetes may be able to degrade PCDD/F congeners more efficiently. However, chances of fungal bioaugumentation for *in situ* bioremediation is very less, since the white-rot fungi are generally unable to compete with native bacteria in soil owing to their slow growth rate and specific growth requirements. Hence, large scale studies or field trials are a must to establish the potential application of fungal bioremediation of dioxins contaminated soil.

Recently, genetically engineered microbes and enzymes have attracted attention for their potential role in bioremediation (Chen *et al*., 1999; Furukawa, 2000; Pieper and Reineke, 2000; Timmis and Pieper, 1999). One of the areas for research under this theme is to expand the substrate range of dioxygenases by mutagenesis of the catalytically active subunit so that they can degrade the wider range of dioxin-related compounds. Recently, dioxygenases of various bacteria have been engineered to enhance their potential for environmental pollutant degradation. The mutagenic techniques used to achieve this include *in vitro* gene shuffling where chimeric genes are produced by mixing dioxygenase genes of different bacteria or the exchange of domain between dioxygenases of different bacteria (Furukawa, 2000). Such engineered enzymes have novel and wider substrate specificity, such as polychlorinated biphenyls, trichloroethylene, and dioxins.

## 11 Conclusion

The bacterial dioxygenase system is the most well studied enzyme system in the field of dioxin degradation. There are reports that dioxygenase can attack either limited numbers of congener or sometimes can lead to incomplete degradation of some of the congeners. Consequently, this reduces the application of these bacteria as bioremediation agents. The fungal peroxidase, laccase, and lignolytic enzymes have broader substrate specificity as compared to the dioxygenase system. Hence, as compared to bacteria, fungi are better candidates for bioremediation, in terms of the broader substrate range. But the major problem with fungi, specifically the white rote fungi or basidiomycetes, is their noncompetitive nature. These are slow growing and are unable to compete with indigenous microorganisms in the contaminated sites, which reduces their capability as bioremediation agents.

The third possibility is the use of genetically engineered microorganisms as potential bioremediation agents. As discussed above, by selective mutagenesis in the active site of dioxygenase enzymes substrate, specificity can be enhanced, and these genetically engineered bacteria grown in the lab can be directly applied in the contaminated site for bioremediation. This process of applying non-indigenous microorganisms for bioremediation is known as a bioaugmentation. One of the major disadvantages of bioaugmentation is

survival of non-indigenous microorganisms. It has been reported that often the non-indigenous microorganisms are unable to compete with indigenous microorganisms. Therefore, survival of genetically modified microorganisms with enhanced dioxin-degradation capability is uncertain. Another promising approach is the use of a microbial consortium. Employing two, three, or more (more than one) dioxin-degrading microorganisms leads to complete mineralization of dioxin congeners. But one major problem with the use of a consortium is growing culturing all the microorganisms together as a consortium and, specially, it is extremely difficult to fulfill all the requirements of microorganisms in an open environment (in contaminated site) outside the lab. Furthermore, competition with indigenous microorganisms is another major cause of concern. Therefore, biostimulation is always a better option as compared to bioaugmentation. In biostimulation, indigenous dioxin-degrading microorganisms in contaminated environments are stimulated to degrade dioxin. This stimulation is done by adding nutrients in the soil. Since these are indigenous bacteria, therefore, there is no issue of competitive survival. The only problem with biostimulation is an absence of dioxin degrading bacteria in the contaminated site. Therefore, further studies are required for successful bioremediation of dioxin contamination. Very few studies have been done on anaerobic degradation of dioxin, which is a very slow process. Thus, there is not of much use in for bioremediation, but a combination of both anaerobic and aerobic approaches might be successful. Hence, further research on microorganisms/enzymes possessing wide substrate specificity and better survival in non-indigenous environments is still required.

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