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DIOXINS AND DIOXIN-LIKE COMPOUNDS IN MEAT AND MEAT PRODUCTS

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Eyad Aoudeh[1], Emel Oz[1], Mohammad Rizwan Khan[2], Fatih Oz[1]*
[1] Department of Food Engineering, Faculty of Agriculture, Ataturk University, Erzurum, Turkey
[2] Chemistry Department, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia

Abstract
Dioxin and dioxin-like compounds are persistent organic pollutants that received considerable attention in recent years due to their high potential toxicity, wide distribution and extreme stability. Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) mainly occur in the environment as a result of several human activities including combustion, incineration and many other industrial activities, whereas polychlorinated biphenyls (PCBs) congeners were intentionally manufactured and widely used in various fields. Since dioxin and dioxin-like compounds are found in various environmental compartments (air, water, soil, sludge, sediment, food, feed, blood, animal and human tissues), humans could be exposed to them via inhalation, dermal contact or food ingestion. However, 90% of human exposure to dioxin is through food ingestion particularly foods from animals and foods that are rich in fat. In contrast, only low levels have been found in food items of plant origin. Exposure to dioxin compounds is associated with various adverse health problems. However, their toxicity varies dramatically according to the type of dioxin, species of exposed organism, as well as exposure frequency and duration. Dioxins are mainly determined by instrumental chromatographic methods such as GC-HRMS and GC–MS/MS. Many efforts have been made to remove, reduce and prevent these hazardous substances from the environment. However, the best method for reducing human exposure to dioxins and dioxin-like compounds is controlling and minimizing their production. In this article, structures, sources, exposure, toxicity and analysis methods of dioxin and dioxin-like compounds in meat and other foods were reviewed.

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Introduction

Persistent organic pollutants (POPs) are the organic compounds that remain in the environment for a relatively long period of time and resist different types of degradation. POPs were reported to cause adverse effects on the environment, animal and human health. The presence of these compounds in the environment occurs as a result of both natural processes and human activities (unintentional or intentional). POPs are often semi-volatile compounds and show hydrophobic properties so they could be transported long distances via atmosphere and tend to bioaccumulate in food chain (especially fatty tissues). Human exposure to POPs occurs through diet and contact with other environmental compartments [1,2]. POPs are categorized into two main groups including both the polycyclic aromatic hydrocarbons (PAH) and some halogenated hydrocarbons.

Dioxins and dioxin-like compounds, known as a major group of POPs, are a group of polyhalogenated aromatic hydrocarbon compounds that share similar chemical structures and properties. They are highly toxic chemicals that are associated with harmful effects to humans and animals, widespread, found almost everywhere in the environment throughout the world being persistent pollutants that have long half-lives and remain in the environment for long periods of time. Generally, dioxins and dioxin-like compounds are poorly soluble in water and have strong lipophilic properties, so they tend to accumulate in adipose tissues of humans and animals. These compounds are mainly formed as a result of human activities such as chemical production and incomplete combustion processes [3–5].

Dioxins released into the environment as a result of washing soda ($NaCO_3$) production in a German chemical plant were characterized for the first time in 1827, but they were not identified until the 1980s [6]. Another action resulted in releasing huge amounts of dioxins to the environment was the use of the herbicide called "Agent Orange" that contained small amounts of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). It was intensively used by the U.S. military during the Vietnam War and resulted in spraying of 150 kg of 2,3,7,8-TCDD over southern Vietnam. Other accidents of dioxin pollution, such as the Times Beach, Love Canal and Seveso disasters were also reported. In 1976, in a town called Seveso located in the north of Italy, an explosion at the chemical factory known as Industrie Chimiche Meda Societa` Anonima (ICMESA) occurred and resulted in releasing chemicals including

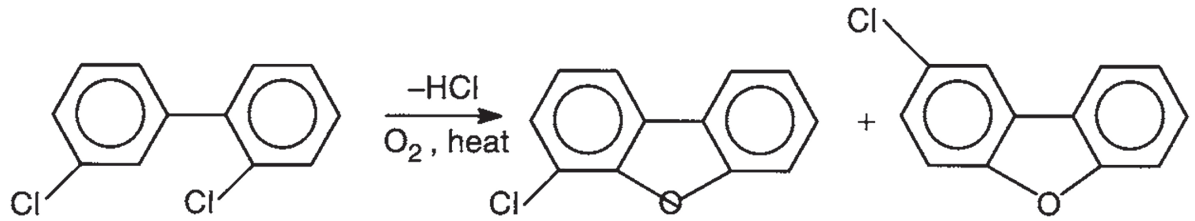

Figure 1. Thermochemical generation of the PCDFs from PCBs [13]

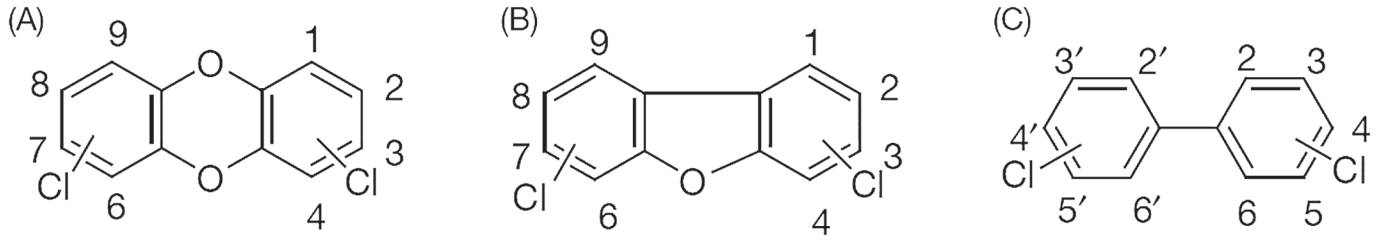

Figure 2. The general chemical structures of PCDDs (A), PCDFs (B) and PCBs (C) [8]

2,3,7,8-TCDD and contaminating an area of 2.8 km^2 with a huge dose of dioxins [7,8].

The term "dioxin" is usually used to refer to polychlorinated dibenzo-p-dioxins (PCDDs) and sometimes used to refer to the most toxic compound (2,3,7,8-TCDD) produced by human beings [5,8–10]. The dioxin family could be classified into two main categories: polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) [10]. In addition, polychlorinated biphenyls (PCBs) are referred to as dioxin-like compounds since some coplanar congeners exhibit dioxin-like toxicity (binding the aryl hydrocarbon "Ah" receptor) and have similar features with dioxins. Moreover, heating PCBs in the presence of O_2 can lead to PCDFs formation (Figure 1); additionally, close relationships between the formation mechanisms for PCBs, PCDDs and PCDFs were also reported [11–13].

The general chemical structures of PCDDs, PCDFs and PCBs are shown in Figure 2. As seen from the figure, PCDFs and PCDDs are polyhalogenated tricyclic aromatic molecules that contain two benzene rings joined by either one or two oxygen molecules, respectively. On the other hand, PCBs consist of two benzene rings directly connected to each other. Each hydrogen atom in the rings could be substituted by a halogen atom such as chlorine [14,15].

Pure dioxins are colorless solids. They are lipophilic, lowly soluble in water, have the low vapor pressure, high melting point. They are extremely stable substances against acids, bases and high temperatures (< 600 °C) [5,10,16,17]. Depending on the number of chlorine substitutions (regardless of the position of chlorine atoms), 10 homologues of PCBs and 8 homologues of PCDDs and PCDFs could be found [1,5]. According to both position and number of chlorine atoms in the rings, 75, 135 and 209 possible congeners of PCDDs, PCDFs and PCBs could be found (Table 1). In fact, not all of these congeners reported to have dioxin-like toxicity and their toxicity degrees are not equal but in turn depend on the degree and the pattern of chlorine substitution on the benzene rings. Lateral substituted congeners with ≥4 chlorine atoms at 2, 3, 7 and 8 positions are reported to have toxic effects [8,13,14].

Table 1. Possible number of PCDD, PCDF and PCB congeners [8,19]

Number of chlorine atoms	Number of congeners		
	PCDD/ PBDD	PCDF/ PBDF	PCB
Mono	2	4	3
Di	10	16	12
Tri	14	28	24
Tetra	22	38	42
Penta	14	28	46
Hexa	10	16	42
Hepta	2	4	24
Octa	1	1	12
Nona	0	0	3
Deca	0	0	1
Total	75	135	209

In addition to the chlorinated dioxins, brominated structural analogues (polybrominated dibenzo-p-dioxins — PBDDs and polybrominated dibenzofurans — PBDFs) could also be formed as a result of the substitution of chlorine atoms by bromine ones (Figure 3). Similar to chlorinated analogues and according to the number and position of bromine atoms, 75 and 135 congeners of PBDDs and PBDFs, respectively, could be found (Table 1). The brominated analogues have similar physicochemical properties and biotoxicity (or even more toxicity) compared to chlorinated dioxins. Brominated dioxins have higher molecular weights, lower vapor pressures, lower water solubility and higher melting points than chlorinated ones. Incineration of electronic waste is considered the main source for brominated dioxins since they contain polybrominated diphenyl ethers (PBDEs) that are regarded as an important precursor of PBDDs and PBDFs [18,19].

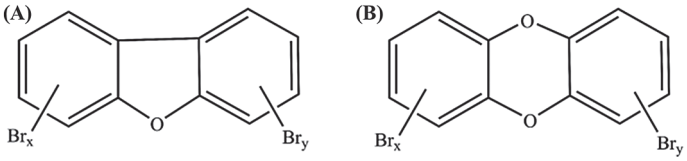

Figure 3. Chemical structures of PBDDs (B) and PBDFs (A) [18]

Sources of dioxins and dioxin-like compounds

The anthropogenic sources are the main sources of dioxins. Only a tiny part of dioxins is of natural origin. Actually, there is no commercial production of PCDDs and PCDFs (except production for research usage), they are formed as an unintentional by-product of some chemical and industrial processes such as herbicides industry (e. g., 2,4,5-trichlorophenoxy acetic acid), metals recycling and industry, pulp bleaching and synthesis of some common solvents (e. g., perchloroethylene-PCE and trichloroethylene-TCE). Dioxins are mainly released into the environment by incineration and combustion processes. In addition, dioxins could be formed during some natural processes such as forest fires, volcanic eruptions, geological processes and biological processes [6,8,10,13,17,20]. However, natural sources of dioxin release a relatively smaller amount of dioxins compared to that released from human activities [17].

Many classifications of dioxin sources can be found in the literature. While Kulkarni [8] categorized the major sources of dioxins into four main groups including incineration, combustion, industrial, and reservoir processes, the U. S. Environmental Protection Agency grouped these sources into five major classes including combustion, metals smelting, refining, and processing, chemical manufacturing, natural sources and processes and reservoirs [21]. Figure 4 summaries the main sources of dioxins according the U. S. Environmental Protection Agency. Dwyer and Themelis [22] grouped PCDDs and PCDFs emission sources into two categories: controlled industrial and open burning sources. The controlled industrial sources include waste-to-energy, waste incineration, electricity and heat generation, metallurgical processes, cement and asphalt production. Whereas the open burning sources include backyard barrel burning, agricultural burning, construction debris, yard waste and fires (forest, vehicle, landfill, building). U.S. EPA also grouped dioxin sources depending on the time between their formation and releasing to the environment into two categories which include contemporary formation sources (dioxins formed and immediately released to the environment) and reservoir sources (previously formed dioxins and dioxin-like compounds are stored in these sources, then they are re-released to the environment) [21].

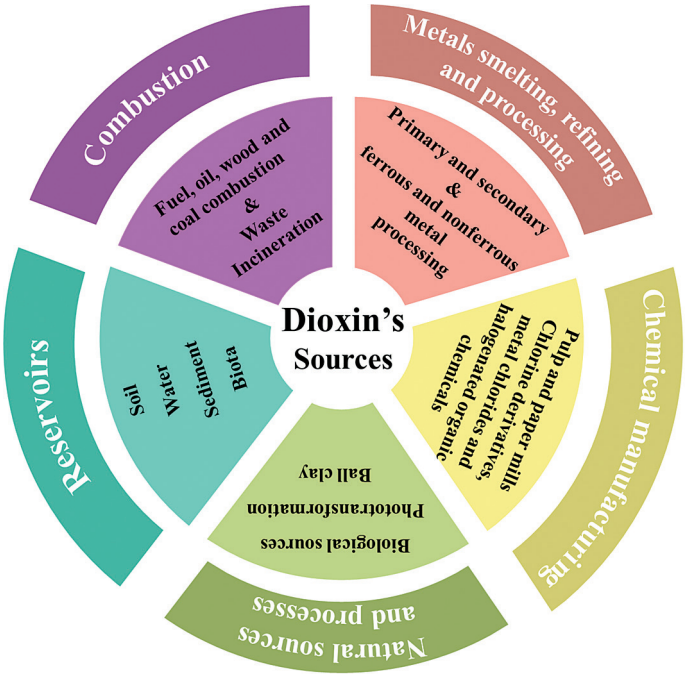

Figure 4. Main sources of dioxin substances

Dioxins and furans occur in most of the combustion and incineration processes Kulkarni et al. [23] indicated incineration processes as the main source of PCDDs and PCDFs generation and release to the environment including municipal solid waste, medical waste, hazardous wastes and sewage sludge incineration processes. In 2012, Dwyer and Themelis [22] reported that the electricity and heat generation processes (which include combustion of wood, coal, gasoline, diesel and other fuel oils) as the major dioxin source that is responsible for 66.2% of the total dioxin emission in the USA. Dioxins are also formed during manufacturing bleached pulp and paper by chlorination of phenolic compounds found in wood pulp and during metal smelting, refining and processing [8]. PCDDs and PCDFs can also be unintentionally formed during manufacturing of some insecticides (e. g., DDT), herbicides (e. g., 2,4-D and 2,4,5-T), disinfectants (e. g., hexachlorophene) and chlorinated aliphatic compounds (e. g., PVC) [21,24]. Finally, PCDDs and PCDFs could be also formed from chlorinated phenolic substances by microorganisms under specific environmental conditions or by photolytic radical reactions of highly chlorinated phenols [8,21].

On the other hand, PCBs occur in the environment intentionally a result of their commercial production or unintentionally as a by-product of combustion and industrial processes [1]. PCBs were commercially produced since 1929 by chlorination of biphenyl in many countries over the world. Due to their unique physical and chemical properties, manufactured PCBs (usually containing congener mixtures with different chlorination degrees) have been extensively used in various industrial and commercial applications including electrical transformer and capacitors (as dielectric fluids), heat transfer systems (as a heat-conduction fluid), hydraulic systems (as a hydraulic oil) and pesticides (as an extender). PCBs were also used in flame retardants, carbonless copy paper, oil-based paints, lubricants, plastics, inks and waterproofing compounds [1,21]. PCBs are also formed as by-products in combustion (e. g., fossil fuel and biomass), incineration (e. g., waste), thermal industries (e. g., ferrous and nonferrous metal smelting) and production of some commercial chemicals (e. g., pigments, 2,4-dichlorophenoxyacetic acid) [11,25–27]. PCBs production was then banned in the late 1970s because of their adverse effects on the environment and human health. However, because of their chemical stability, broad usage and their inadvertent production, PCBs are considered important persistent environmental pollutants [1,21].

Kanan and Samara [2] in their review mentioned that major sources of dioxin and dioxin-like compounds varied across countries, i. e., while the fuel combustion for generation of electricity and heat was reported as the principal source of these compounds in the United State [22], burning the household garbage was responsible for the largest dioxin emissions in Canada. In Turkey, the contamination of aquatic organisms was associated with industrial discharges, whereas the highest dioxin emissions resulted from waste incineration in Spain, France and Italy. Higher concentrations of dioxins were reported in the urban areas when compared to those at remote areas in the Middle East. Similarly, samples collected from the high industrial activity areas exhibited high amounts of dioxins [2].

Exposure to dioxins and dioxin-like compounds

Dioxin and dioxin-like compounds are usually released to the environment in form of mixtures, which vary widely in their individual congener content and proportions depending on a source. They can also be changed over time, transported over long distances apart from production or releasing areas or redistributed within environmental media [14,21]. After releasing dioxins and dioxin-like compounds from their sources, they are distributed to most environmental media and can move between these media. They have been detected in air, water, sediment and soil. The wide presence of dioxins in the environment is mainly related to physical and chemical properties especially persistency and bioaccumulation [2,21,28]. Dioxins, which are formed by incineration and combustion processes, accidental release and explosions, are responsible for the presence of dioxins in the atmosphere. Whereas they enter soil and water from industrial discharges or deposition of atmospheric dioxins. Dioxins can also enter water as a result of contact with contaminated soil. Due to their hydrophobic properties, they tend to be associated with the organic matrix instead of being dissolved in water; consequently, they deposit in sediment with a very low concentration in the dissolved phase. Dioxins movement from water or soil to the air is less common because of their low vapor pressures [2,10,14]. Booth et al. [29] estimated that 57% and 40% of annual dioxin emission is deposited to soil and ocean water respectively, whereas 3% of it remained in the air. On the other hand, EFSA [16] revealed that dioxin-like PCBs contribute to about 63% of human exposure, whereas PCDD and PCDF groups account for 14% and 23%, respectively. Regarding the individual congeners, PCB-126 had the highest contribution (54.7%) to the total human exposure, followed by 2,3,4,7,8-PeCDF (10.7%), 1,2,3,7,8-PeCDD (7.4%), 2,3,7,8-TCDF (4.9%), PCB-169 (3.7%) and 2,3,7,8-TCDD (3.4%).

Humans are exposed to dioxin and dioxin-like compounds via inhalation, dermal absorption, ingestion of contaminated soil attached to fruit or vegetables and consumption of contaminated food. With the exception of accidental and occupational exposures, inhalation and dermal contact are not considered important pathways of human exposure. However, more than 95% of human exposure to dioxins is attributed to food consumption, particularly food of animal origin [14,16,21]. The total dietary exposure of the European population to 29 congeners of dioxins (17 PCDD/Fs) and dioxin-like (12 PCBs) compounds were estimated to be between 0.4 and 2.6 pg WHO_{2005}-TEQ/kg body weight (bw)/day. Furthermore, it was reported that the estimated dietary exposures of toddlers and children were almost two-fold higher than those estimated in teenagers and adults [16]. Due to the persistency and lipophilic features of dioxins and dioxin-like compounds, they are known to concentrate and bioaccumulate in adipose tissues of animals, fish and humans. Additionally, dioxins can be excreted from edible products containing fat such as milk and eggs. Some specific congeners also tend to accumulate in the liver. Generally, foods with higher fat content (especially, animal fats) tend to have higher levels of dioxins [30]. Consequently, food items such as meat, dairy products, fish and eggs (especially seagull eggs, fish liver and offals) are most likely to contain high concentration of dioxins [8,16,17]. Moreover, organisms with higher trophic levels are known to contain high levels of dioxins due to their biomagnification through the food chain [14]. Table 2 shows the levels of total dioxins and dioxin-like compounds in some food groups collected from different regions and during different periods. As seen from the table, dioxin compounds are reported in all foods categories, including fruit and vegetables. However, only foods from animal origin show high levels of dioxins. The authors also reported differences in the dioxin levels in food items within the same group. For example, EFSA [31] indicated that salmon fish had the highest level of dioxins compared to the other fish species and kinds of seafood. Anonymous [30] also reported that fatty fish such as salmon, full-fat cheese, butter and high-fat beef had higher levels of dioxins than other food items within the same category. On the other hand, zucchini was reported to have higher levels of dioxins due to the fact that, unlike other plants, zucchini and pumpkin belonging to the genus *Cucurbita* are able to absorb dioxins from contaminated soils and translocate them to the other parts of the plant, including the fruit [32].

Regarding the contribution ratio of different foods to human exposure to dioxins, it is reported that consuming contaminated fish accounts for 30–75% of total human exposure to PCDD/Fs and PCBs [34]. EFSA [16] indicated that the consumption of fatty fish contributed up to 56% of total human exposure to dioxins, whereas cheese and livestock meat consumption contributed up to 21.8% and 3.8%, respectively, of the total exposure. However, the contribution ratio of food items to human exposure does not only depend on their contamination level, but the consumption frequency among the population is also considered an important factor [16]. The percentage contribution of different sources and food items to human exposures to dioxins and dioxin-like compounds is summarized in Figure 5.

Table 2. Levels of PCDD/Fs and PCBs in some food groups

Food Group	pg TEQ/g	pg TEQWHO98/g	pg WHO05-TEQ/g
Meat and meat products	0.005–0.46	1.97	0.105 (0.003–2.067)
Poultry & poultry products	0.004–0.06	—	0.068 (0.007–0.782)
Fish, seafood & their products	0.01–0.33	4.42	0.284 (0.005–12.365)
Hen eggs	0.01–0.05	1.01	0.052 (0.011–0.202)
Milk	0.0006–0.01	1.49	0.030 (0.003–0.149)
Dairy products	0.0001–0.24	1.29	0.087 (0.002–0.505)
Fats & oils	0.002–0.22	—	0.090 (0.010–0.305)
Nuts	0.003–0.006	—	0.020 (0.014–0.024)
Cereals & cereal products	0.0001–0.05	—	0.018 (0.002–0.050)
Fruit	0.0007–0.01	—	0.005 (0.001–0.027)
Vegetables	0.0001–0.05	—	0.007 (0.001–0.295)
Region	US	Europe	Taiwan
Period of time	1999–2001	1999–2008	2004–2018
Other	Unspecified	Results based on ww, except for fish based on fat	Based on wet weight (ww)
Reference	[30]	[31]	[33]

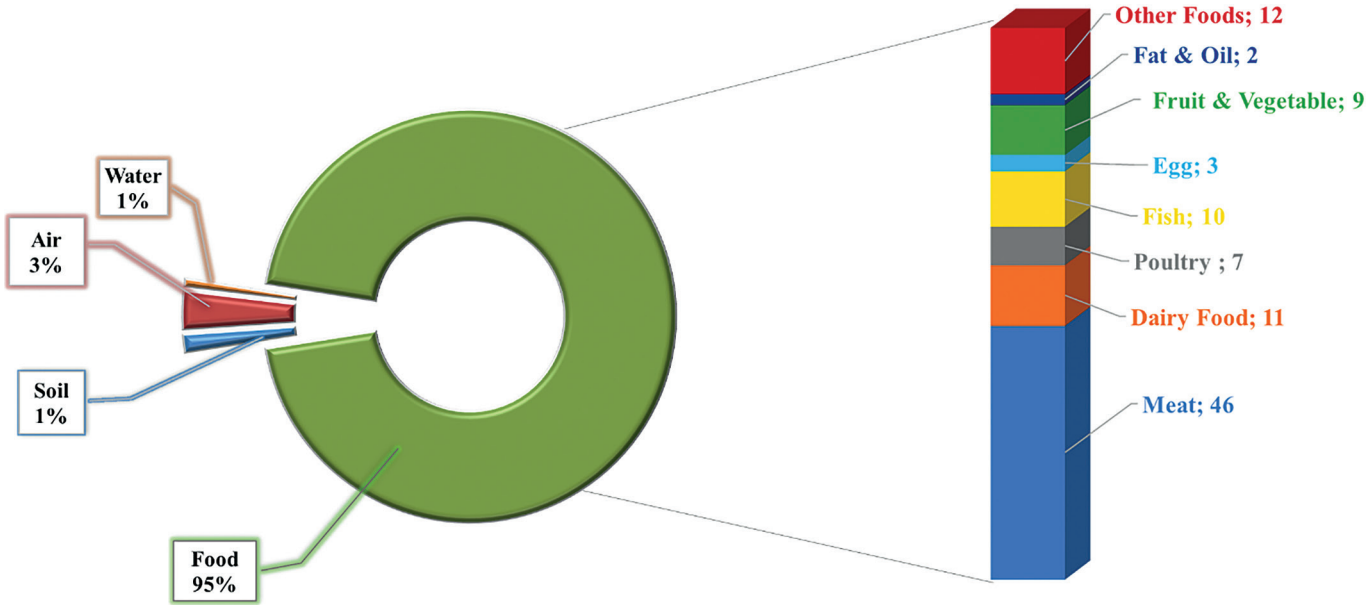

Figure 5. Contribution (%) of different sources and food items to human exposures to dioxins and dioxin-like compounds [24,30]

It is worth mentioning that food processing can lead to significant losses of dioxin compounds. Lower levels of PCDD/Fs and PCBs were observed in the processed food compared to the raw ones. Lower chlorinated congeners could be released during cooking when high temperature is used. Dioxin intake could also be minimized during food processing by removing fat from food. Otherwise, using contaminated cooking oil during cooking results in processed products with higher amounts of dioxin compared with raw materials [16]. Planche et al. [35] reported significant losses (18–48%) of PCBs in meat as a result of pan cooking; the losses also increased with increasing the intense of cooking conditions. However, no significant losses in PCDDs and PCDFs amounts were observed. Hori et al. [36] reported that grilling or boiling mackerel slices reduced the levels of PCDD/Fs by 31% or 14%, respectively. Whereas the reduction in beef slices was about 42% when treated by boiling. Domingo [37] in his review indicated that cooking processes that caused reducing or eliminating fat from food led to a decrease in the concentration of some contaminants like as PCDD/Fs and PCBs.

Toxicity of dioxins and dioxin-like compounds

Dioxin and dioxin-like compounds have attracted considerable interest throughout the world due to their potential high toxicity. They had received public attention in 1976 when the highest known exposure to dioxins mainly TCDD happened as a result of releasing a huge amount of toxic chemicals to the environment by an explosion at ICMESA plant in Seveso, Italy [8,38]. Several studies demonstrated the reverse health effects of dioxins on several organs and systems in both humans and animals [16]. It is reported that the toxicity of dioxin compounds strongly depends on the dioxin type i. e., the substitution degree and pattern [2]. As we mentioned previously, there are 75, 135 and 209 possible congeners of PCDDs, PCDFs and PCBs, respectively. But only PCDD/Fs congeners that are halogenated (chlorine or bromine) at 2, 3, 7 and 8 positions; and only coplanar congeners of PCBs that are substituted with ≥4 chlorine (or bromine) atoms are considered toxic. Thus, only 29 congeners of dioxin and dioxin-like compounds (7 PCDDs, 10 PCDFs, and 12 PCBs) exhibit dioxin-like toxicity (compounds are shown in Table 3) [16,21,39]. 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is considered the most toxic and widely investigated congener. Furthermore, the International Agency for Research on Cancer (IARC) has classified 2,3,7,8-TCDD as carcinogenic to humans (Group 1) [40]. However, oral reference dose (RFD) for TCDD is defined as the dose that is probably to be without considerable risk of adverse health effects over a lifetime. It was estimated by the US EPA in 2012 as 0.7 pg TCDD/kg bw per day. Similarly, the minimal risk level (MRL) for chronic oral exposure to 2,3,7,8-TCDD was determined by the Agency for Toxic Substances and Disease Registry (ATSDR) as 1.0 pg/kg bw/day [16]. The 29 toxic congeners of PCDD/Fs and PCBs not only exhibit the harmful effect at low doses but also possess relatively long half-lives in the human body that vary depending on the type of dioxins. For instance, the half-lives of 2,3,7,8-TCDD, PCB-126 and 2,3,4,7,8-PeCDF are estimated to be 6.5, 1.6 years and 7.0, respectively [16,40,41]. In addition to the dioxin type, the frequency and duration of exposure are also important to determine their toxicities. In order to express the toxicity of different dioxins and dioxin-like compounds, the World Health Organization (WHO) created the toxic equivalency factor (TEF) system. In this system, the toxicity of dioxin and dioxin-like congeners was compared to the toxicity of the most toxic member (2,3,7,8-TCDD). Thus, the TEF value given to TCDD is 1.0 and the other compounds have TEF values relative to TCCD and ranging from 1 to 0.00001 (Table 3). In other words, TEF values express the possible toxicity of certain congeners relative to TCDD (the reference congener). Regarding the toxicity of the dioxin mixture (since they are released to environment in a form of mixture), a total toxic equivalency (TEQ) is used and calculated by multiplying the concentration of each congener by its TEF value, then the products are summed. TEF values have been revised and developed many times since their establishment. In 2005, the World Health Organization (WHO) introduced the final TEF values and proposed separated values for mammals, birds, and fish since different species show different sensitivities to specific dioxin members. The current TEFs are termed WHO_{2005}-TEFs. Previously proposed TEF values were termed WHO_{1998}-TEQ, I-TEQs and Nordic-TEQs. These TEFs were used to express a dioxin level in many studies particularly those performed before the year 2005. So, it is important to be aware of which TEF values were used when evaluating dioxin levels or exposures. Unlike WHO_{1998}-TEQ and WHO_{2005}-TEQ, I-TEQs and Nordic-TEQs do not include the TEF values for dioxin-like compounds (PCBs) [8,14,16,21].

Table 3. Toxic equivalency factors (TEFs) for PCDDs, PCDFs and dioxin-like PCBs [42].

	Congener	WHO_{1998} TEF	WHO_{2005} TEF
PCDDs	2,3,7,8-TCDD	1	1
	1,2,3,7,8-PeCDD	1	1
	1,2,3,4,7,8-HxCDD	0.1	0.1
	1,2,3,6,7,8-HxCDD	0.1	0.1
	1,2,3,7,8,9-HxCDD	0.1	0.1
	1,2,3,4,6,7,8-HpCDD	0.01	0.01
	OCDD	0.0001	0.0003
PCDFs	2,3,7,8-TCDF	0.1	0.1
	1,2,3,7,8-PeCDF	0.05	0.03
	2,3,4,7,8-PeCDF	0.5	0.3
	1,2,3,4,7,8-HxCDF	0.1	0.1
	1,2,3,6,7,8-HxCDF	0.1	0.1
	1,2,3,7,8,9-HxCDF	0.1	0.1
	2,3,4,6,7,8-HxCDF	0.1	0.1
	1,2,3,4,6,7,8-HpCDF	0.01	0.01
	1,2,3,6,7,8,9-HpCDF	0.01	0.01
	OCDF	0.0001	0.0003
Non-ortho PCBs	3,3',4,4'-tetraCB (PCB 77)	0.0001	0.0001
	3,4,4',5-tetraCB (PCB 81)	0.0001	0.0003
	3,3',4,4',5-pentaCB (PCB 126)	0.1	0.1
	3,3',4,4',5,5'-hexaCB (PCB 169)	0.01	0.03
Mono-ortho PCBs	2,3,3',4,4'-pentaCB (PCB 105)	0.0001	0.00003
	2,3,4,4',5-pentaCB (PCB 114)	0.0005	0.00003
	2,3',4,4',5-pentaCB (PCB 118)	0.0001	0.00003
	2',3,4,4',5-pentaCB (PCB 123)	0.0001	0.00003
	2,3,3',4,4',5-hexaCB (PCB 156)	0.0005	0.00003
	2,3,3',4,4',5'-hexaCB (PCB 157)	0.0005	0.00003
	2,3',4,4',5,5'-hexaCB (PCB 167)	0.00001	0.00003
	2,3,3',4,4',5,5'-heptaCB (PCB 189)	0.0001	0.00003

When dioxins and dioxin-like compounds enter the human body, they are distributed to the liver and fatty tissues as well as blood lipids. Most of these compounds are poorly metabolized and have long half-lives in the human body. They vary depending on type and level of

dioxin, age, body mass index (BMI) and gender. PCCD/Fs and dioxin-like PCBs are associated with several health impacts including chloracne, endocrine disruption, immune system disorder, reproductive disorder and cancer [8,16]. The acute toxicity of dioxins has been associated with the development of chloracne that is considered to be the clearest and most specific sign for the dioxin toxicity. However, this condition is only observed in high exposure cases such as accidental or occupational exposure [10,16]. Other adverse health effects such as irritation in the respiratory and gastrointestinal tracts, headache and fatigue can also be a result of acute or short-term exposure to dioxins. Moreover, many animal studies indicated adverse effects on many organs such as the thymus and adrenal glands, liver and heart. However, the toxicity of these compounds varies dramatically between animal species. For example, the oral LD_{50} values (the dose responsible for killing 50% of the exposed animals) for TCDD are 0.6 μg/kg for guinea pigs, 20 μg/kg for rats and 1175 μg/kg for hamsters [10]. Concerning health risks related to chronic exposure to dioxins, many studies indicated the association between dioxin exposure and the disruption of hepatic function, immune disorder, cardiovascular problems, reproductive disorders and cancers, even though there are no sufficient data to confirm this association. Studies on rats exposed to relatively low levels of TCDD showed reduction in sperm production, delayed puberty, hepatic implications and alteration in bone parameters [10,16]. The toxicity mechanism of dioxins and dioxin-like compounds is complicated and not completely elucidated. However, toxic congeners of PCDD/Fs and PCBs are believed to have a common mechanism of toxicity by disrupting the function of the aryl hydrocarbon (Ah) receptor. They are generally known as Ah-receptor ligands. Most dioxin congeners can bind the Ah-receptor that acts as a transcription factor and controls genetic transcription from DNA to RNA [8,10]. The binding affinity of dioxin compounds to the Ah-receptor varies between different congeners and different species. For example, the binding affinity of the Ah-receptor to TCCD in humans is lower than that in rats and mice [16]. The toxicity of PCDD/Fs and PCBs is initiated by binding to the Ah-receptor. Accordingly, the inappropriate and continuous activation of this receptor subsequently induce the production of several proteins, especially cytochrome P450 1A1, which, in turn, can affect the metabolism of important substances such as steroid hormones, leading to several changes and disorders in biological functions and cellular processes [8,14].

Dioxins are suspected to be associated with immunosuppression by affecting the development of T-cells in the thymus [14]. Many studies reported a correlation between parents' exposure to dioxins and the incidence of allergies and infections in their children during childhood [16]. Additionally, immunosuppression by dioxin exposures is suggested as the reason of mass fatalities of seals and dolphins in the 1980s in Europe [14]. However, EFSA [16] reported insufficiency in the available evidence to confirm the relationship between exposure to PCCD/Fs and dioxin-like PCBs and negative effects on the immune system in adults or children.

PCCD/Fs and dioxin-like PCBs are widely known to adversely affect the function and development of the reproductive system (particularly, in males) based on the results of both animal and human cohort studies [16]. Experimental animal studies reported the abilities of TCDD and some dioxin compounds to reduce the number of estrogen receptors, interrupt the testosterone hormone and affect the development of the prostate. Thus, it is thought that the alteration of sex hormone levels in serum is the possible mechanism to explain the final adverse reproductive symptoms [10,14]. However, the Panel on Contaminants in the Food Chain (CONTAM Panel) in the European Food Safety Authority (EFSA) did not consider the hormonal changes, per se, in adults and children to be a causal negative effect of dioxin exposures [16]. Animal studies on rats, mice and other rodents indicated symptoms such as reduction of sperm synthesis, sperm count and postponement of sexual maturity in males; deformation in the urogenital system, irregularity of the estrous cycle and reduction of the ovulatory rate in females due to exposures to dioxins, particularly, TCDD [14,16]. Semen quality, cryptorchidism and development of puberty are affected adversely by dioxin exposures. The CONTAM Panel in the EFSA indicated a causative relationship between exposure to PCDD/Fs (especially TCDD) during infancy or before sexual maturation and reduced semen quality. This relation depends on the results of both experimental animal studies and human prospective studies including those performed after the Seveso accident [16]. On the other hand, there are limited evidence to support a causative relation between exposure to PCDD/Fs or PCBs and both cryptorchidism (undescended testicle) and postponed puberty. A reduction in the gender ratio (lower probability of male birth) in offspring of males exposed to high levels of 2,3,7,8-TCDD (accidentally or occupationally) has been indicated in many cohort studies [43–45]. This reduction was suggested to be causal by the expert team of ESFA [16]. However, no changes were observed in the gender ratio in offspring of exposed females in the same studies. Additionally, the association with other birth outcomes such as low birth weight, preterm birth could not be proven by the available studies. Concerning the adverse effects on the female reproductive system, no relationship was observed between exposure to dioxins and female pubertal development. The existing studies also did not provide sufficient evidence to associate dioxin exposures with effects such as endometriosis, altered menstrual cycle, altered ovarian function and changes in time of menopause [16].

Again, the existing studies did not show enough evidence to prove the causative adverse effects of dioxin exposures (including TCDD congeners) on the thyroid function or disorders [16]. However, a causative relation was

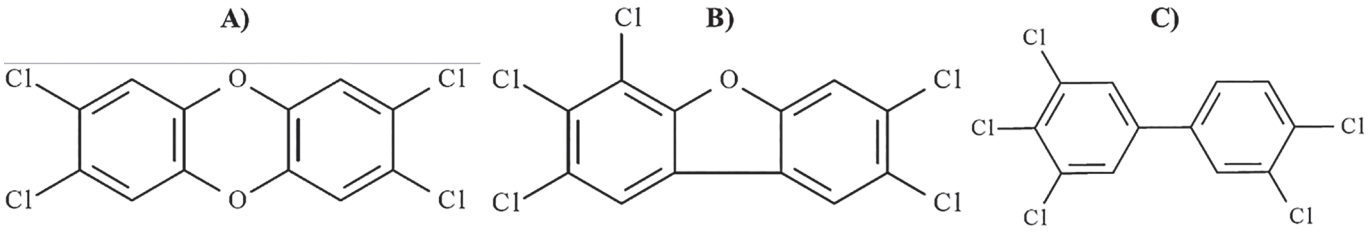

Figure 6. The chemical structures of 2,3,7,8-TCDD (A), 2,3,4,7,8-PeCDF (B) and PCB-126 (C) [40].

observed between children born to mothers exposed to high levels of 2,3,7,8-TCDD in Seveso and increased concentrations of TSH in the serum of newborns [46], whereas no adverse effects were reported with low-moderate exposure to dioxin and dioxin-like compounds including 2,3,7,8-TCDD [47,48].

Regarding the cardiovascular risk as a result of dioxin exposures, an increment of the risk has only been indicated with occupational exposure to very high levels of 2,3,7,8-TCDD (serum TCDD > 1,000 pg/g fat) [49], whereas exposure to relatively lower levels of TCDD or other dioxin congeners was either associated with increased cardiovascular risk [50,51] or not [52,53].

Even though epidemiological studies proposed a possible relationship between exposure to PCDDs and PCDFs and hepatic dysfunction, the EFSA expert team concluded that hepatic diseases were not causally associated with exposure to PCDDs and PCDFs due to the insufficient evidence from these studies [16]. EFSA [16] suggested a dose-related and causal association between childhood exposure to PCCD/Fs, particularly TCDD, and enamel defects or hypomineralisation. Similarly, exposure to dioxin and dioxin-like compounds is suggested to be related to changes in bone parameters such as mineral density, size and strength. Finally, positive association was found between occupational, accidental or environmental exposure to toxic PCDD/Fs and PCBs and all cancers combined. They can promote tumors in experimental animals at many sites such as skin, ovary and liver. However, there is no obvious link to any certain cancer site [16,40]. Due to the sufficient evidence obtained from both epidemiological and experimental animal studies as well as the common mechanism of action; 2,3,7,8-TCDD, 2,3,4,7,8-Pentachlorodibenzofuran (2,3,4,7,8-PeCDF) and 3,3′,4,4′,5-Pentachlorobiphenyl (PCB-126) (Figure 6) were classified as carcinogenic to humans (Group 1) by IARC [40].

It is worth mentioning that the WHO determined the tolerable daily intake as 1–4 pg TEQ/kg body weight/day for dioxin and dioxin-like compounds, whereas it was reported as 2 pg TEQ/kg bw/day in the United Kingdom [8,9,16].

Analysis methods of dioxins and dioxin-like compounds

The analysis of dioxin and dioxin-like compounds is crucially demanded due to the high toxicity and widespread occurrence of these compounds in different environmental and biological matrixes, in addition to the need to monitor their levels in these matrixes and control their releases from sources. Since they are usually found in a very low concentration (at levels of pg/g or fg/g) as congener mixtures and attached/adsorbed to other organic compounds, analytical methods should provide the efficient extraction, purification, separation and accurate determination of toxic congeners at trace levels [9,32,54]. Thus, the analytical methods for dioxin and dioxin-like compounds determination are required to have high sensitivity, selectivity, and specificity, as well as high accuracy and precision with the low limit of detection (LOD) and limit of quantification (LOQ) [9].

To assess compliance with various legislation and regulations, many analytical methods have been developed for dioxin detection and determination. They are mainly determined by instrumental chromatographic methods usually coupled to mass spectroscopic- or bioassay-based methods that are mainly used to determine dioxins in environmental specimens. Chemical and biological methods commonly used for determination of dioxins substances are summarized in Figure 7. Bioassay-based methods have a strong probability to differentiate between the more stable congeners (Ah-receptor ligands) and the other dioxin congeners. Unlike the chromatographic methods, bioassay-based methods generally have lower costs, are fast and, thus, allow handling a relatively larger number of samples. On the other hand, they are considered semiquantitative methods, their results (some methods) are expressed as Bioanalytical Equivalents (BEQs) and results that exceed the cut-off-level need to be re-analyzed using confirmatory methods. Since chromatographic methods are able to identify individual dioxin compounds and provide their exact concentration, they are considered confirmatory methods (gas chromatography/high-resolution mass spectrometry "GC-HRMS" and gas chromatography/tandem mass spectrometry "GC–MS/MS"). However, there is a good correlation between the results obtained by bioassays methods and those obtained by chromatographic ones such as GC-HRMS and GC–MS/MS [16,32,55,56].

Bioassay-based methods used for dioxin determination depend on screening specific responses resulted from organisms or cells when exposed to dioxins, or the capability of some receptors, enzymes, antibodies or any other biological molecules to identify the structural property of dioxin and dioxin-like compounds [55,56]. They could be grouped into *in vivo* and *in vitro* assays. *In vivo* bioassays are based on experimentally exposing the laboratory animals to dioxin compounds and investigating the response

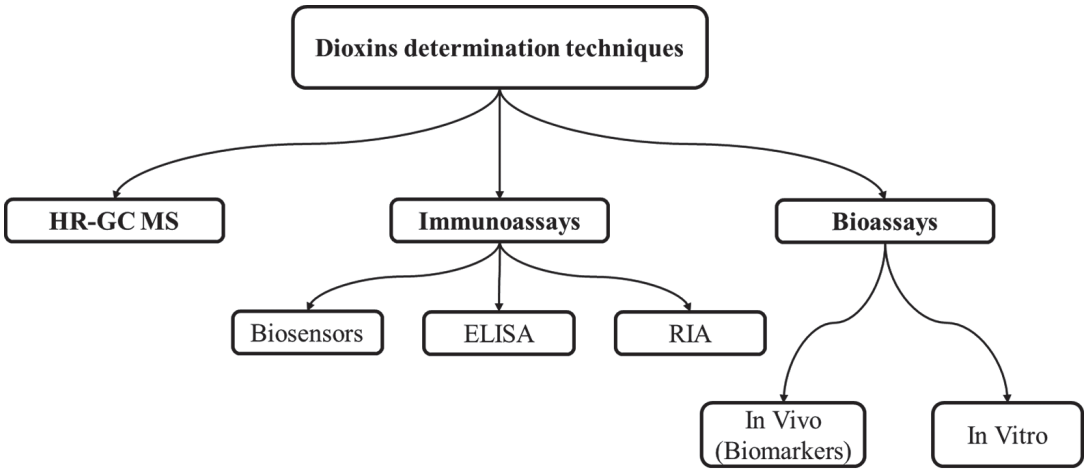

Figure 7. Chemical and biological techniques for determination of dioxins

or the resulted abnormality in different organs such as thymus for immune toxicity and liver hepatotoxicity, or assessing some *in vivo* biomarkers of natural exposure to dioxin in humans or wildlife (e. g., cytochrome P450 1A gene- CYP1A and induction of aryl hydrocarbon hydroxylase-AHH). Whereas, the *in vitro* bioassays include methods based on DNA-binding, receptor binding, cell culture and reporter gene assays. In addition, changes in gene expression or enzyme inhibition assays in cultured cells and several immunoassay-based methods, particularly, fluorescence immunoassay, the enzyme-linked immunoassay and radioimmunoassay were also applied to determine dioxin compounds [55,56].

The Chemical Activated LUciferase gene eXpression (CALUX) is the most widely used bioassay for dioxin and dioxin-like compounds detection. It uses genetically modified hepatoma cells that contain the Ah-receptor responsive luciferase reporter gene. This gene reacts to any substance which can stimulate the Ah-receptor (including dioxins), so exposing these cells to dioxins leads to induction of luciferase gene expression and consequently increases the luciferase levels that can be measured by light reaction. Since, the CALUX analysis responds to all chemicals that activate the Ah-receptor, adding a clean-up step over an acid silica column could decrease the interfering compounds and increase the specificity of the analysis for the PCDD/Fs and DL-PCBs. Ethoxyresorufin-O-deethylase (EROD) assay is another bioassay analysis for dioxin and dioxin-like compounds determination but it is less commonly used [16,32,57].

Chemical instrumental methods used for dioxin and dioxin-like compounds determination by chromatographic analysis include gas chromatography (GC) and high-performance liquid chromatography (HPLC) coupled with different types of detectors such as mass spectroscopy, high-resolution mass spectroscopy, fluorescence spectroscopy, electron capture detector or photodiode array [55,56]. Many techniques were reported for separation and detection of dioxin and dioxin-like compounds such as two-dimensional gas chromatographic (GC×GC) separation, GC–MS/MS, GC–HRMS, high-resolution gas chromatography coupled to mass spectrometry (HRGC–MS), high-resolution gas chromatography-high resolution mass spectrometry (HRGC–HRMS), GC coupled to electron capture detector (GC-ECD) and gas chromatography (GC) triple quadrupole mass spectrometry (GC–QQQMS/MS). High-performance liquid chromatography coupled to a photodiode array (HPLC-PDA) was also used for dioxin analysis [9,55,56,58]. However, only methods fulfilling the criteria laid down by the European Commission are considered as confirmatory methods. These methods should provide clear congener-specific identification and quantification of dioxin and dioxin-like compounds in the samples and they usually use GC–HRMS and GC–MS/MS. Moreover, the CONTAM Panel in the EFSA only included the data obtained with GC–HRMS, GC–MS/MS, HRGC–HRMS, GC–HRMS or GC–QQQ-MS/MS in their Comprehensive European Food Consumption Database [16]. Each step in the analysis process is crucial in order to reduce the interferences from other compounds and to avoid analyte losses. Therefore, it is important to pay attention during all stages of the analytical process including sampling, handling of samples, extraction, clean-up, separation, detection, and quantification [9].

First, representative samples should be collected with equipment precleaned by acetone or hexane and/or heat-treated at 450 °C for 20 min. Lipid determination is a critical step in the analysis of dioxin compounds, as the internal standard should be added before the fat extraction in the samples of food and feed of animal origin that contain less than 10% fat, whereas, it could be added prior or later fat extraction in those that contain more than 10% fat [16]. Because of the hydrophobic nature of the dioxin and dioxin-like compounds, extraction methods are based on fat extraction from the samples including liquid-solid extraction (e. g., Soxhlet, accelerated solvent extraction-ASE, microwave-assisted solvent extraction-MAE and supercritical fluid extraction-SFE), solid-phase extraction (SPE), and liquid-liquid extraction (LLE). However, an extraction method is chosen depending on the sample type, amounts and the nature of other interfering substances. Another critical step in the analysis process is extract purification

that provides eliminating interfering substances. Dioxins, as stable compounds, could be cleaned-up from other interfering compounds (e. g., protein and fat) by treating with a strong acid such as sulfuric acid and/or a base. Gel permeation chromatography (GPC) has been also used in dioxin extract purification. However, multistep purification using chromatographic adsorbents (silica, florisil, alumina, and activated carbon) is routinely applied for the isolation of dioxin from other interfering substances [16]. Finally, the $^{13}C\text{-}_{12}$ labelled standards (17 $^{13}C\text{-}_{12}$ labelled PCDD/Fs congeners and 12 $^{13}C\text{-}_{12}$ labelled PCBs) are used as internal standards to determine the losses (recovery) of their corresponding analytes [32].

Conclusion

Dioxin and dioxin-like compounds have received considerable attention in recent years, especially after many accidental events that led to releasing huge amounts of these compounds to the environment. They attracted attention and became familiar chemicals between populations due to the high potential toxicity to humans and other organisms, wide distribution over the world and extreme stability. PCDDs and PCDFs mainly occur in the environment as a result of several human activities such as combustion, incineration and many other industrial activities. A very small amount is also reported from some natural processes such as volcanic eruptions and forest fires. Unlike PCDD/Fs, the PCB congeners were intentionally manufactured and widely used in various fields. Huge amounts of PCBs were produced in the period 1929–1970, but their production was suspended in the late 1970s because of their adverse effects on the environment and human health. Once dioxin and dioxin-like compounds are released from their sources, they are spread almost everywhere throughout the world and enter various environmental compartments (air, water, soil, sludge, sediment, food, feed, blood, animal and human tissues) via direct or indirect ways. Humans are exposed to dioxin via inhalation, dermal contact or food ingestion. However, 90% of human exposure to dioxin is through food ingestion particularly foods from animals and foods that are rich in fat. In contrast, only low levels of dioxin and dioxin-like compounds are found in food items of plant origin. These compounds show various adverse health effects started from chloracne, irritation in the respiratory and gastrointestinal tracts, headache to serious problems in the reproductive, immune, thyroid, cardiovascular and hepatic function. They can also promote many types of cancers. However, the toxicity of dioxin and dioxin-like compounds varies dramatically according to species of exposed organisms and the type of dioxin i. e., a degree of chlorine substitution and pattern, moreover, the exposure frequency and duration are also important factors. These health effects were documented based on many experimental animal studies and human cohort or epidemiological studies. However, some of these health effects are not supported by sufficient evidence that confirms the causal association between dioxin exposures and health problems. Regarding dioxin type, only 29 congeners of a total of 419 PCDDs, PCDFs and PCBs are reported to show toxic effects on humans and many other living organisms. Their toxicities are expressed as the TEF value that exhibits the possible toxicity of certain congeners to a reference congener (TCDD- the most toxic congener). TEF values were developed many times and different versions of TEFs were used in different studies. Several methods have been developed to measure dioxins in environmental and biological samples, since they usually occur as a mixture of congeners at very low concentrations and are often attached to other organic compounds. So, analytical methods should provide an efficient extraction, purification, separation and accurate determination of toxic congeners at trace levels. Dioxins are mainly determined by instrumental chromatographic methods or bioassay-based methods. The latter are generally fast, have lower cost, allow to handle a relatively large number of samples but they are considered semiquantitative methods, so their results need to be confirmed by other confirmatory methods such as GC-HRMS and GC–MS/MS. Because of the high toxicity, wide distribution, accumulation ability, poor degradation and stability for a very long period of time, many efforts have been made to remove, reduce and prevent these hazardous substances from the environment. The best method for reducing human exposure to dioxins and dioxin-like compounds is prevention and minimization of production and contamination of foods and animal feeds. Moreover, processing food, sometimes, can lower the concentration of PCDD/Fs and PCBs in food items by discarding the fat during the process or releasing lower chlorinated congeners. Trimming fat from meat, consuming low-fat dairy products and avoiding foods from contaminated areas could also minimize the exposure to dioxin and dioxin-like compounds. Under certain conditions, dioxin and dioxin-like compounds in the environment undergo biodegradation by both aerobic and anaerobic organisms. Highly chlorinated PCDD/Fs and PCBs are dechlorinated via anaerobic organisms, whereas aerobic organisms are responsible for mineralization of the resulted less-chlorinated compounds. Many microorganisms including yeasts, fungi and bacteria are able to degrade dioxins. However, this process strongly depends on the position and degree of chlorine substitution, the species of microorganisms and the status of the medium. Various methods have also been developed to reduce emission of dioxin and dioxin-like compounds in fly ash and flue gases released from incineration and combustion processes including the particulate matter collection, scrubbers or spray absorber, sorbent or flow injection process for flue gases, thermal treatment, non-thermal plasma, UV irradiation for fly ash.

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AUTHOR INFORMATION

Eyad Aoudeh, MSc, PhD Candidate, Department of Food Engineering, Faculty of Agriculture, Atatürk University, 25240, Erzurum, Turkey. Tel.: +904–42–231–26–44, E-mail: eyad.aoudeh151@ogr.atauni.edu.tr
ORCID: https://orcid.org/0000–0002–0097–8450

Emel Oz, PhD, Associate Professor, Department of Food Engineering, Faculty of Agriculture, Atatürk University, 25240, Erzurum, Turkey. Tel.: +904–42–231–16–25, E-mail: emel.oz@atauni.edu.tr
ORCID: https://orcid.org/0000–0003–3766–2713

Mohammad Rizwan Khan, PhD, Professor, Chemistry Department, College of Science, King Saud University, 11451, Riyadh, Kingdom of Saudi Arabia. Tel: +966–011–467–41–98, E-mail: mrkhan@ksu.edu.sa
ORCID: https://orcid.org/0000–0001–6270–8539

Fatih Oz, PhD, Professor, Department of Food Engineering, Faculty of Agriculture, Atatürk University, 25240, Erzurum, Turkey. Tel.: +904–42–231–26–44, E-mail: fatihoz@atauni.edu.tr
ORCID: https://orcid.org/0000–0002–5300–7519
* Corresponding author

All authors bear responsibility for the work and presented data.

All authors made an equal contribution to the work.

The authors were equally involved in writing the manuscript and bear the equal responsibility for plagiarism.

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