

Chapter 1

Introduction

1.1 Introduction

The presence of dioxins in the environment has attracted considerable attention in recent years from the public and scientific community. These compounds are not intentionally produced, but a variety of industrial and combustion have been identified as the sources. The Bangkok Post, June 10, 1999 reported that high contamination of dioxins had been found in imported meat, eggs and dairy products from Belgium. As a consequence the Government imposed a ban on animal feed and meat from Belgium. The Livestock Department's of Thailand was also closely monitoring the imported meat from Belgium and its neighbours. It was also collaborating with the Commerce Ministry, the Thai Embassy in Belgium, the Food and Drug Administration as well as quarantine stations all over the country to keep abreast of the situation. Later on June 14, the Ministry of Agriculture banned all materials from Belgium used in the production of animal feed, such as animal fat, meat, and bones. The Department would maintain the ban and strict inspection until the situation improves. Furthermore, certification to verify product safety was required for feed and premix imported from Belgium's neighbouring countries, i.e. France, the Netherlands and Germany. The contamination of cancer-causing dioxin had induced Belgium's neighbours to ban Belgian pork products. The Netherlands announced a ban on Belgian pork. The German state of North Rhine-Westphalia also urged butchers not to slaughter Belgian pigs. Thailand also banned Belgian meat, poultry and dairy products after it was revealed that dioxins had been found in samples of Belgian animal feed. Particular concern surrounded the question of which brands of milk powder were safe or unsafe for babies and small children to consume. The scare over the presence of dioxin in key food products was only the latest in a series of controversies over the way food was produced and its impact on human health and the environment (Khor, 1999).

Many studies investigated the acute lethality of PCDD to animals and there are in shown Table 1.

Table 1 LD₅₀ of dioxins on various species

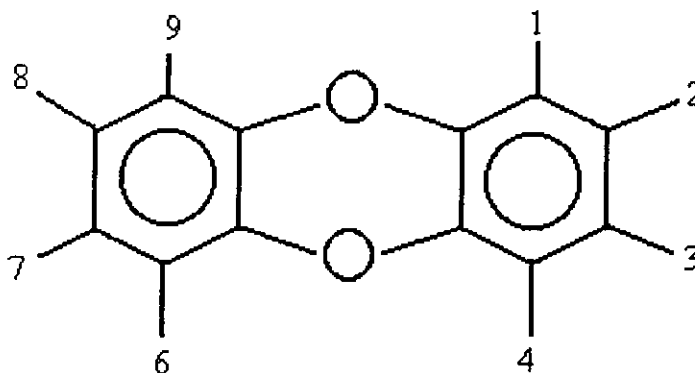
Source	Species	Amount of LD ₅₀ of dioxins
Schwetz <i>et al.</i>	Guinea pig	0.6 µg/kg (2,3,7,8-TCDD)
McConnell <i>et al.</i>	Guinea pig	2.0 µg/kg (2,3,7,8-TCDD)
	Rhesus monkeys	70.0 µg/kg (2,3,7,8-TCDD)
	Mice	284.0 µg/kg (2,3,7,8-TCDD)
	Guinea pig	31.0 µg/kg (1,2,3,7,8-5CDD)
	Guinea pig	125.0 µg/kg (1,2,4,7,8-5CDD)
	Guinea pig	30,000 µg/kg (2,3,7-3CDD)
Leger <i>et al.</i>	Guinea pig	0.6 mg/kg (2,3,7,8-TCDD)
	Mice	300 mg/kg (2,3,7,8-TCDD)
	Frogs	300 mg/kg (2,3,7,8-TCDD)
Karasek <i>et al.</i>	Guinea pig	300,000 µg/kg (2,8-2CDD)
		29,000 µg/kg (2,3,7-3CDD)
		1 µg/kg (2,3,7,8-4CDD)
		3 µg/kg (1,2,3,7,8-5CDD)
		1,125 µg/kg (1,2,4,7,8-5CDD)
		73 µg/kg (1,2,3,4,7,8-6CDD)
		100 µg/kg (1,2,3,6,7,8-6CDD)
		100 µg/kg (1,2,3,7,8,9-6CDD)
		7,200 µg/kg (1,2,3,4,6,7,8-7CDD)
	Mice	4,000 mg/kg (1,2,3,4,6,7,8,9-8CDD)

The difficulty of determining precisely the toxicity of dioxins is reflected in the fact that almost every individual country has its own norms defining the recommended Toxic Daily Intake (TDI) (Table 2). Polychlorodibenzo-*p*-dioxins, PCDDs are constituent members of related families of molecules containing an aromatic phenyl core and various chlorine substitutions (Figure. 1) and are chemically classified as halogenated aromatic hydrocarbon. PCDDs have a triple-ring structure that consist of two benzene rings connected by a third oxygenated ring. And the benzene rings are connected by a pair of oxygen atoms.

Table 2 Toxic Daily Intake (TDI) of dioxins from various source

Source	Amount of TDI of dioxins
World Health Organisation (WHO)	10 pg/kg/day
Natherlands	4 pg/kg/day
Japan	10 pg/kg/day
US EPA	Less than 0.006 pg/kg/day
NOAEL (no observable adverse effect level)	10 ng/kg/day
LOAEL (lowest observable adverse effect level)	100 ng/kg/day

The best known, the extremely toxic tetratogenic and mutagenic of the dioxins is 2,3,7,8 tetrachlorodibenzo *-p-* dioxin, TCDD (Cattabeni *et al*, 1976). A total of 75 different dioxins compounds are possible, having from 1 to 8 chlorine substitutents.

Figure 1 The structure of Polychlorodibenzo *-p-* dioxins, PCDDs

Some works have been done to study Physical properties of dioxins such as Marple *et al.*, (1986) reported the water solubility of 2,3,7,8,-TCDD as 19.3 ± 3.7 ng/L at 20°C . Rordorf (1989), using a gas-flow method in a saturation oven, reported the vapor pressure data for 2,3,7,8-TCDD as 1.50×10^{-9} mm Hg at 25°C . Burhard and Kuehl (1986) used reversed-phase High Pressure Liquid Chromatography and Liquid Chromatography/Mass Spectrometry for detection and determined the octanol/water partition coefficients for 2,3,7,8-TCDD and a series of seven other tetrachlorinated planar molecules. The $\log K_{ow}$ values for the four TCDD isomer ranged from 7.02 to 7.20. The $\log K_{oc}$ values for 2,3,7,8-TCDD have

been measured by Lodge and Cook (1989). As reported, contaminated sediments from Lake Ontario and distilled water in glass cylinders were used to measure $\log K_{oc}$ and this was found to be in the range of 7.25 to 7.59.

Polychlorinated dibenzo-p-dioxins (PCDDs) are persistent and toxic compounds, which are formed as by-products in several different industrial processes such as chlorinated phenols, phenoxy herbicides, and PCBs and combustion processes such as municipal incinerators, wood burning, chemical waste combustion, automotive emissions, pathological waste, and PCB-filled transformer fires. Thus, they are distributed all over the world and some certain areas are highly contaminated with PCDDs. For example, in Finland over 250 sawmills are contaminated with PCDDs due to the previous use of a wood preservative, Ky-5, which is a chlorophenol formulation containing PCDDs as impurities.

The toxic nature of dioxins at extremely low concentrations poses a very difficult problem for scientists and regulators to address. Typical analytical detection limits for water samples collected using conventional sampling methods range from one to ten parts per quadrillion (ppq). The US EPA, however, established a water quality criterion for the protection of human health for 2,3,7,8-tetrachlorodibenzo-p-dioxin (the only dioxin congener with a water quality standard) at 0.013 ppq (EPA, 1984). Due to the limitations of the analytical methods, a result of the inability to accurately quantify dioxin concentrations in surface waters that were at levels of concern, an alternative sampling method for concentrating dioxin from large volumes of water was necessary.

Amberlite XAD-2 polymeric adsorbent is a hydrophobic crosslinked polystyrene copolymer resin. The extremely hydrophobic nature of the resin attracts other hydrophobic organic compounds such as dioxins (Supelco, 1995). So in this work Amberlite XAD-2 was used as an adsorbent for the pre-concentration of dioxins in water sample. After concentrating on the adsorbent, dioxin is extracted by ultrasonic extraction, optimized to replace the traditional liquid based methods (Soxhlet extraction) in routine analysis of polychlorinated dibenzo-p-dioxins (PCDDs) in solid (Dinkins, 1998). Ultrasonic extraction has successfully been used for non-polar compound extraction of solids and it has overcome the disadvantages of traditional extraction of PCDDs, more economical, minimization of manual labour and faster extraction methods. The analysis would then be done using a Gas Chromatography – Mass Spectrometry technique. This is universally accepted as the

instrumental technique for qualitative and quantitative analysis of dioxins in environmental samples. The identification of dioxin is based on retention times and of the responses at the M^+ and $(M+2)^+$ values on the Selected Ion Monitoring (SIM) chromatogram (Hashimoto *et al.*, 1995).

1.2 Literatures review

Dioxins and furans are two families of related chemical compounds known as polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. Similar in chemical structure and biological properties, dioxins and furan are usually found together in the environment as complex mixtures.

Much of this concern has focused on the most toxic member of the dioxin and furan family. This was the contaminant in the weedkiller 2,4,5-TCDD, also used by the U.S. military in Agent Orange, and in the oil sprayed on the unpaved roads of Times Beach, Missouri, in the early 1970s. Only 17 of all 210 dioxins and furans have four lateral chlorine atoms. Table 3 lists the 17 compounds and their toxic equivalency factors relative to 2,3,7,8-TCDD. These factors are called the International Toxicity Equivalency Factors (I-TEF) and were developed by scientific experts in several countries (Ministry of Environment and Energy, 1997).

Effect on animals

Extensive laboratory animal testing has shown that 2,3,7,8-TCDD can cause a number of serious health problems: 1) weight loss, 2) skin disorders, 3) immune system damage, 4) impaired liver function, 5) altered blood function, 6) impaired reproduction/birth defects, 7) increased incidence of tumors and 8) increased enzyme production (Ministry of Environment and Energy, 1997).

Effects on humans

Studies have been done on people who have been accidentally exposed to high levels of dioxins and furans at work, as a result of improper waste disposal, or from eating contaminated cooking oil. Chloracne, a skin disorder, is the most common human health effect. Extreme exposures also lead to other effects on the skin, liver, immune system, reproduction system, senses and behavior. The effects of long-term, low-level human exposure are still being investigated. Current scientific evidence does not indicate a link

between normal, everyday exposure and long-term health effects such as cancer, coronary disease and impaired reproduction (Ministry of Environment and Energy, 1997).

Table 3 The International Toxicity Equivalency Factors (I-TEF)

Dioxins*	I-TEF
2,3,7,8-Tetrachlorodibenzo-p-dioxin	1.0
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	0.5
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	0.1
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	0.1
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	0.1
1,2,3,4,6,7,8-Heptachlorodibenzo-p-dioxin	0.01
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	0.001
2,3,7,8-Tetrachlorodibenzofuran	0.1
2,3,4,7,8-Pentachlorodibenzofuran	0.5
1,2,3,7,8-Pentachlorodibenzofuran	0.05
1,2,3,4,7,8-Hexachlorodibenzofuran	0.1
1,2,3,6,7,8-Hexachlorodibenzofuran	0.1
1,2,3,7,8,9-Hexachlorodibenzofuran	0.1
2,3,4,6,7,8-Hexachlorodibenzofuran	0.1
1,2,3,4,6,7,8-Heptachlorodibenzofuran	0.01
1,2,3,4,7,8,9-Heptachlorodibenzofuran	0.01
1,2,3,4,6,7,8,9-Octachlorodibenzofuran	0.001

* The other 193 dioxins and furan have negligible toxicities in comparison and are assigned a value of zero (Ministry of Environment and Energy, 1997).

Quality guideline standard

Ministry of Environment and Energy (1997), Canada reported the quality guideline standard. It was possible to calculate tolerable levels of exposure for all dioxins and

furans. This was done by taking the concentrations of the 17 most toxic dioxins and furans, multiplying each one by a toxic equivalency factor (TEF)-its toxicity relative to 2,3,7,8-TCDD- and adding up all the corrected concentrations expressed as 2,3,7,8-TCDD toxic equivalents (TEQ) (Ministry of Environment and Energy, 1997). There are 3 types of sample quality guideline as shown in Table 4 (Ministry of Environment and Energy, 1997).

Table 4 Ontario's standards for dioxins

Types	Criterion	Amount
Air	Ambient air quality criterion (24 hours)	5 pg TEQ / cubic metre
Drinking water	Interim maximum allowable concentration	15 pg TEQ / litre
Surface water	Residential soil remediation criterion	1000 pg TEQ / gram
	Agricultural soil remediation criterion	10 pg TEQ / gram

Analytical Methods

There are some studying of dioxins residue in various types of sample such as sediment, soil, natural water as following :

Dioxins contaminated in sediments and soil samples were analyzed from the water collected from nine sampling points in the Baltic Sea selected to be representative of background levels (Broman *et al.*, 1992). The concentration of particle associated total dioxins/furans varied between 0.170 and 0.390 pg/L with an average concentration of 0.230 pg/L. The total dioxins/furans concentration of dissolved fraction varied between 0.036 and 0.260 pg/L with an average concentration of 0.120 pg/L. In another report Ohsaki *et al.*, (1995) studied PCDD/Fs in the sediment samples from reservoir and irrigation ponds, and in soil from Paddy Field by using ¹³C-labeled internal standards and a selected ion Monitoring Method of High Resolution Gas Chromatography Mass Spectrometry. These compounds were found in all samples analyzed. The average concentrations of the total PCDD/Fs in the 10 sediment samples taken from reservoir ponds were 83, 3900, and 360 pg/g of dry weight, respectively, those in some sediments from irrigation ponds were 96,

3200 and 96 pg/g, those in some sediments from an irrigation pond near an incineration facility were 260, 10000, and 290 pg/g, respectively, and those in soil from a paddy field near an incineration facility were 120, 96000, and 4700 pg/g, respectively. These findings suggest that one of the pollution sources of PCDD/Fs was municipal waste incineration.

Dioxins contaminated in natural water have also been studied. Lohmann *et al.* (2000) studied the contamination of dioxins in Raritan Bay and Hudson River Estuary near the New York-New Jersey. Dioxins dissolved in the 60L water sample from the two sources were 2940 and 2350 fg / L, respectively. Rantalainen *et al.* (2001) reported on the other reports of dioxins in water include 100 fg / L in Japanese coastal sea and 210 – 280 fg / L in River Elbe, Germany (Kutz *et al.*, 1995)

Mechanisms of the entry of dioxins/furans into the aquatic food chain is also an interesting topic. Smith *et al.*, (1995) analyzed CDD/CDF concentrations in sediment cores, air, precipitation, soil, and stormwater runoff in an effort to determine the contributing sources of these compounds to the lower Hudson River. The mass balance estimates developed from these data are: stormwater runoff entering tributaries (76%); anthropogenic wastes (19%); atmospheric deposition (4%); and shoreline erosion (less than 1%). Physical properties of dioxins is another topic of interest. Marple *et al.*, (1986) studied that the water solubility of 2,3,7,8,-TCDD which was found to be 19.3 ± 3.7 ng/L at 20°C.

Because dioxins are extremely toxic compound, its degradation process is interesting in Miller *et al.* Miller and Zepp (1987), Choudry and Webster (1987) and Atkinson (1996) reported that photolysis appears to be one of the most environmentally significant degradation mechanisms for CDD/CDFs in water and soil. CDD/CDFs adsorb electromagnetic radiation at wavelengths greater than 290 nm and, therefore, can be expected to be subject to photolysis by sunlight.

The concentration of dioxins residue found is normally in the ultra-trace level, So sample preparation is important for improvement. There are several types of technique depending on the type of sample. The first type of sample to concern is sample with particle. This was studied by Taylor *et al.* (1995) who reported on sample preparation from effluent samples by solid phase extraction disks (SPE Disks). Several types of filtering media were layered on top of the SPE Disks to overcome the prefiltering system i.e. allowed an entire sample extraction. Dioxins were then directly eluted from

the filtering medium and SPE Disks. The mean values of the internal quantitation standard recovery ranged from 58 to 70 % for liquid-liquid extraction and from 65 to 77 % for SPE Disks. The average recovery for SPE method was 8 % higher than the liquid-liquid extraction.

In another work sample preparation for the analysis of dioxins residue in water was done by using Adsorbent, Amberlite XAD-2, (Hashimoto *et al.*, 1995) and 1.3 – 2.9 pg/L of dioxins were found in seawater of Japanese areas, extracted from XAD-2 resin column. The dioxin detected was mostly OctaCDD with some HeptaCDDs. To be able to analyze the trace amount of dioxin large volume of water is needed. Dinkins *et al.* (1998) reported a high volume of water sample, 1000 liters, was needed to quantify the ambient water column concentration on the Ohio and Kanawha Rivers. In their process the water sample, 1000 liters, was filtered over a twelve-hour period. Three of the fourteen samples had on undetectable levels of 2,3,7,8 TCDD in dissolve phase, the detection limit was below 0.001 ppq.

1.3 Objectives

- To obtain the sample preparation technique suitable for the qualitative and quantitative analysis of dioxins residue in water by Gas Chromatography – Mass Spectrometry (GC – MS)
- To analyze dioxins residue from water sample in contaminated area