

Evaluation of Dioxins and Polychlorinated Biphenyl in Growing Sheep's, Bovines and Fish's Meat Using Chromatography Techniques

¹Abed J. Kadhim, ²Fadhil M. Abid and ¹Jasim M. Salman

¹Al-Nisour University College, Baghdad, Iraq

²Ministry of Science and Technology, Baghdad, Iraq

Abstract: Polychlorinated dibenzo-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs) are commonly referred together as “dioxins.” The quantity of dioxins released in to the environment from regulated industrial sources has significantly declined in the last 30 years due to effective pollution controls. Today, backyard burning of trash is the largest source of dioxin emissions to the US environment. Thus, dioxins/furans are largely a legacy pollution issue. The important of analyzing samples for dioxins/furans in water, soil, sediment, tissue and air has been widely introduce in environmental pollution. Persistent organic compounds dioxins and furans are persistent organic compounds that are by-products of natural and industrial processes. Dioxins are chemically stable and are found throughout the environment. They accumulate in the food chain, mainly in the fatty tissue of animals. The most studied form of dioxin is the 2, 3, 7, 8-TCDD isomer. When released into the air, dioxins can be transported long distances and as a result are found around the world. When dioxins are released into water, they tend to settle into sediments where they bio accumulate in fish and other aquatic organisms and biomagnifying in the food chain such that aquatic species have higher concentrations than plants, water, soil and sediments. There are a variety of methodologies available for the analysis of dioxins. Detection limits range from the parts per trillion to parts per quadrillion level. Dioxins/furans-low resolution methods such as SW-846 8280 (water and solids) and EPA 613 (water) are most appropriate for investigation and characterization projects. These methods are applicable to contaminated soil, water and waste and generally have detection limits that are an order of magnitude higher than their high resolution counterparts. Dioxins/furans-high resolution ultra trace level dioxin analysis is available by methods EPA 1613, SW-846 8290, method TO-9 for ambient air and methods 0023A and 23 for source emissions testing. Typically, 17 isomers are evaluated at the part per trillion to part per quadrillion level. Total homologues and/or toxic equivalency factors can also be reported. These methods are most appropriate for drinking water programs, sediment projects, source emissions testing, ambient air monitoring, risk assessments and superfund site investigations.

Key words: Solid core, dioxin, polychlorinated biphenyls, PCB, furans, environment, HPLC

INTRODUCTION

Dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and Polychlorinated Dibenzo Furans (PCDFs)) and Polychlorinated Biphenyls (PCBs) are a family of compounds that share certain chemical structures and biological characteristics and they are toxic chemicals that persist in the environment and accumulate in the food chain (Van den Berg *et al.*, 1998; Berg *et al.*, 2006). They are not produced intentionally but are formed during combustion (burning) processes and as by-products of industrial processes. They have been used in transformers, building materials, lubricants, coatings, plasticizers and inks, although their use has now largely been phased out (Kulkarni *et al.*, 2008; Weber *et al.*,

2008). Both the dioxins and the PCBs are highly resistant to breakdown processes and consequently persist in the environment, followed by uptake into the food chain. Up to 90% of human exposure to dioxins results from the consumption of food containing dioxins, mainly foodstuffs of animal origin with a high fat content, since, these contaminants accumulate in fatty tissues. Foodstuffs in which dioxins can occur include meat, fish, eggs and milk (Galbenu, 2009). Dioxins and PCBs are toxic chemicals that can provoke serious health effects such as cancer, hormone disruption, reduced ability to reproduce, skin toxicity and immune system disorders when exposure to them continues over an extended period (a number of years). Short periods of exposure are not considered to have adverse effects on health, unless the levels are very

high (Breivik *et al.*, 2002). Because of their potentially serious health effects and their persistence in the environment, it is essential to minimize their release into the environment, including the establishment of emission limits for dioxins to air, prohibition of the use of PCBs and safe collection, storage and environmentally compatible disposal or destruction of dioxin and PCB-contaminated devices and products. The dioxin and PCBs compounds tumor that produces a result of the disintegration of pesticides as by secondary burning industrial waste and carcinogenic (Galbenu, 2009; Breivik *et al.*, 2002).

MATERIAL AND METHODS

Reagents: We obtained reagent-grade standards of 2, 2, 4, 5-Tetrachlorinated biphenyl (TCB), 2, 3, 4, 5, 6-Pentachlorinated biphenyl PCB), 2, 2, 3, 3, 6, 6 Hexachlorinated biphenyl (HxCB), 1, 2, 3, 4-Tetrachlorinated dibenzo-p-dioxin (TCDD), 1, 2, 3, 4, 7-Pentachlorinated dibenzo-p-dioxin (PCDD), 1, 2, 3, 4, 7, 8-Hexachlorinated dibenzo-p-dioxin (HxCDD), 1, 2, 3, 4, 6, 7, 8-Heptachlorinated dibenzo-p-dioxin (HpCDD) and Octachlorinated dibenzo-p-dioxin (OCDD) from Ultra Scientific (250 Smith Street, North Kingstown). Stock solutions (PCBs: 5.0, PCDDs: 0.45 ppm) were prepared in toluene for each compound. XAD-2 resin was obtained from Rohm and Haas (Philadelphia, PA). The XAD-2 resin which had been washed and dried was spiked with PCB and PCDDs at a few ng (10^{-9} g) levels. All solvents were HPLC grade from Burdick and Jackson (Muskegon, USA).

Ultrasonic extraction procedure: The Ultrasound assisted Extraction (USE) procedure used was based on a report by (Okamura *et al.*, 1994) with some modification. It was performed using a Fisher Scientific (Pittsburgh, PA, USA) solid state/ultrasonic FS-28 (bath type) and 60 Sonic Dismembrator (50W, probe type). Thus, 50 mL of a liquid solvent (acetone, toluene or their mixture) was added to the sample (10 g of XAD-2 spiked with PCDDs). The mixture was sonicated in an ultrasonic bath for 30 min. The solvent supernatant was transferred to a round flask and the residue was subjected to the same procedure twice. The amount of solvent used was 30 and 20 mL, successively. The collected supernatant was concentrated to about 3 mL. The sonication of the probe type was performed in the same procedure as the above, except for an extraction time of 3 min. Therefore, a 10 g sample was extracted three times with acetone, toluene or a 1:1 mixture of the two for 30 min for the bath type and 3 min for the probe type at about 3°C.

HPLC-UV detection procedure: The amount of PCBs and Dioxins in the liquid extracts was determined on a Shodex C18-5B (250×4.6 mm, 5 μm; Shoko, Kyoto, Japan) column with a 100 μL sample loop. The HPLC system used in this research was a Shimadzu Liquid chromatography equipped with an SPD-10A UV-visible detector and a C-R6A integrator. The injected volume was 20 μL and the flow rate of the mobile phase was 1.0 mL/min. The temperature was 40°C. An acetonitrile/water (93:7, v/v) solution was used to separate the analytic in >30 min. Chromatograms were recorded at 240 nm (A.U.F.S. = 0.005).

Gas Chromatograph-Mass Spectrometer (GC/MS): High Resolution Gas Chromatography combined with a double-focusing High Resolution Mass Spectrometer (HRGC-HRMS) is used. The sensitivity required is 0.1 pg for tetra- and penta-compounds, 0.2 pg for hexa and hepta-compounds, 0.5 pg for octa-compound and 0.2 pg for coplanar PCBs. The GC oven covering 50-350°C to set optimal temperature program of the target compounds. The capillary column is made from fused silica with 0.25-0.32 mm in internal diameter and 25-60 m in length of which inside is coated with strong-polarity liquid of cyanopropyl compound for PCDDs/PCDFs determination. For coplanar PCBs, non-polarity or semi-polarity column of methyl silicone is generally used, recently the column with siloxane- carborane is also used. Mass spectrometer double-focusing type is used. The resolution required is >10,000. The machine can keep ion source temperature at 250-350°C which ionizes with Electron Impact ionization (EI) by 35-70 V of the ionization voltage. Selected Ion Monitoring (or SIM) should be adopted as a detection method. The frequency of the SIM can be adjusted to be <1 sec. Lock-mass method is adopted to detect ions. Injection port should be able to inject the total volume of sample with high reproducibility. For example, split less type and on column type meet the criteria. Helium gas with a purity of >99.999%. Standard reference materials for calibration, compounds for mass spectrometry with high boiling point such as Perfluorokerosene (PFK) (Blomberg *et al.*, 1997; Gaines *et al.*, 1999; Pursch *et al.*, 2002).

RESULTS AND DISCUSSION

Harmful dioxins and Polychlorinated Biphenyls (PCBs) can enter foodstuffs through air, soil or sediments. These substances which contaminate food are therefore, also referred to as environmental contaminants (Esposito *et al.*, 2010). Dioxins have never been specifically produced, they are unintended or undesired

Table 1: Europe-wide action levels and maximum levels for dioxins and dioxin-like PCBs (dl-PCBs) in selected types of food

Type of food	EU action level WHO-TEQ pg/g fat		EU maximum level WHO-TEQ pg/g fat	
	Dioxins	dl-PCBs	Dioxins	Dioxins+ dl-PCBs
Fish				
Meat and meat products	1.25	0.75	3.5	7.5
Oils and fats	1.25	0.75	3.5	7.5
Bovine animals and sheep				
Meat and meat products	1.75	1.75	2.5	4.0
Oils and fats	1.75	1.75	2.5	4
Milk and dairy products including butter fat	1.75	2	2.5	5.5
Animal products				
Mixed animal fats	1.0	0.75	1.5	2.5

PN: Unit of measurement: 1 pg (pico gram) = 0.000 000 000 001 g

by-products of chemical processes in the chlorine-based chemical industry and any combustion process involving chlorine or organic carbon under specific circumstances (such as temperatures between 250 and 800°C and specific residence times) (Kotz *et al.*, 2012). Unlike dioxins, PCBs used to be produced for specific purposes, mainly as non-burning, non-conductive viscous liquids for use in transformers and hydraulics (mining). Since, the mid-1980's, the release of dioxins and PCBs into the environment has been reduced thanks to numerous regulatory measures. Dioxin emissions from known sources dropped by more than 90 between 1990 and 2004. By 2010 values had dropped even further. Today thermal processes of metal production and processing and small combustion systems cause major part of dioxin emissions. As of 1 January 2012 and December 2013, respectively, new legally binding maximum levels (limit values) and voluntary action levels (early warning system) apply across Europe to harmful dioxins and Polychlorinated Biphenyls (PCBs) in various types of food of animal origin, vegetable oils and infant food. In particular, harmonizing previously differing national maximum levels for non-dioxin-like PCBs in food which resulted in tightened provisions as compared to former German limit values and expanding the provisions to also apply to foods for infants and young children for reasons of precaution are a tangible contribution to improving the protection of consumer health and food safety. Table 1 shows The Europe-wide action levels and maximum levels for dioxins and dioxin-like PCBs (dl-PCBs) in selected types of food (Hoogenboom *et al.*, 2015, Malisch and Kotz, 2014).

We measured and determine the amount of dioxin and PCBs in bovine, sheep and fish in the province of Baghdad by the use of techniques are HPLC, GC/MS in order to determine the optimum conditions for standardization and the appointment of limited detection on dioxin. By collecting samples of animal and fish meat from different regions and the results were as shown in the Table 2.

Table 2: The amount of dioxins (PCDDs/PCDFs) and PCBs in growing sheep, bovine and fish meat in pg/g fat by HPLC, GC/MS

Product	Quantity of samples	Dioxins (PCDDs/PCDFs)	Dioxins+ PCBs	Units (pg/g)
Sheep meat	25	3.27	5.12	Fat
Bovine meat	30	2.92	4.78	Fat
Fish meat	29	4.96	8.52	Fat

Table 2 shows the high levels concentration of dioxins (PCDDs/PCDFs) and PCBs in the sampling plan that included different types of meat (sheep, bovine, fish) from different regions of Baghdad. Contaminated for ages and pastures are known to be the major pathways of dioxins and PCB uptake in grazing animals. The relation between polluted soil and contamination in sheep tissues was investigated by (Lindstrom *et al.*, 2005). Feedstuffs are the main input source of dioxins and PCBs into food of animal origin. Due to the, so, called “carry-over effects” these substances turn over from feedstuffs into foods of animal origin and accumulate. For prevention and reduction of these undesirable substances in food reduction in feedstuff is already necessary.

CONCLUSION

So, this research aims to set an optimal method for the standardization of the amount of dioxin in bovine, sheep and fish in the province of Baghdad by the use of techniques are HPLC, GC/MS in order to determine the optimum conditions for standardization and the appointment of limited detection on dioxin by collecting samples of animal and fish meat from different regions and determination of it.

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