LEVELS OF PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) IN HOME AND COMMERCIALLY PRODUCED POULTRY EGGS IN MALAYSIA

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DECLARATION

I hereby declare that this research was sent to Universiti Sains Malaysia (USM) for the degree of Master of Science in Health Toxicology. It has not been sent to other universities. With that, this research can be used for consultation and photocopies as reference.

Sincerely,

ATIQAH BINTI TAHZIZ

(P-IPM0011/18)

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LIST OF SYMBOLS

%	Percent
°C	Degree celsius
<	Less than
±	Plus minus
µL	Microliter
µm	Micrometer

LIST OF ABBREVIATIONS

APC	Antigen presenting cells
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BMF	Biomagnification factor
CE	Collision energy
CV	Coefficient of variation
CXP	Collision cell exit potential
DNA	Deoxyribonucleic acid
DP	Declustering potential
dSPE	Dispersive solid phase extraction
ECF	Electrochemical fluorination
EP	Entrance potential
EPA	Environmental Protection Agency
Fe	Iron
FTCA	Fluorotelomer saturated carboxylate
FTOH	Fluorotelomer alcohols
FUSLE	Focused ultrasound solid liquid extraction
GCMS	Gas chromatography mass spectrometry
HDL	High density lipoprotein
Kg	Kilogram
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LDL	Low density lipoprotein
LLOQ	Lower limit of quantitation
LOD	Limit of detection

m/z	Mass to charge ratio
mg	Milligram
ml	Milliliter
mm	Millimeter
MRM	Multiple reaction monitoring
N_2	Nitrogen
ng	Nanogram
PCBs	Polychlorinated biphenyls
PCDD/Fs	Polychlorinated dibenzo-p-dioxins/furans
PFAS	Perfluoroalkyl substances
PFBA	Perfluorobutanoate
PFBS	Perfluorobutane sulfonate
PFCA	Perfluoroalkyl carboxylic acid
PFCs	Perfluorinated compounds
PFHXs	Perfluorohexane sulfonate
PFNA	Perfluorononanoate
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PFSA	Perfluoroalkyl sulfonic acid
pg	Picogram
POPs	Persistent organic pollutants
PPAR-α	Peroxisome proliferator activated receptor-alpha
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
PSOSF	Perfluorooctane sulfonyl fluoride

PTFE	Polytetrafluoroethylene
QC	Quality control
r^2	Coefficient of determination
RMR	Resting metabolic rate
ROS	Reactive oxygen species
RSD	Relative standard deviation
SPE	Solid phase extraction
TDI	Tolerable dietary intake
TFC	Turbulent flow chromatography
TMF	Trophic magnification factor
ToF	Time of flight
UHPLC	Ultra-high performance liquid chromatography
UPC2	Ultra-performance convergence chromatography
VLDL	Very low density lipoprotein
w/w	Weight by weight

TAHAP KEPEKATAN PEIFLUROOKTANA SULFONAT (PFOS) DAN ASID PEIFLUROOKTANOIK (PFOA) DALAM TELUR POLTRI DARIPADA TERNAKAN DI RUMAH DAN KOMERSIAL DI MALAYSIA

ABSTRAK

Peifluorooktana sulfonat (PFOS) dan asid peiflurooktanoik (PFOA) merupakan dua komponen utama sebatian berfluorin yang digunakan secara meluas dalam industri dan produk konsumer kerana ciri uniknya yang kalis air dan gris. Penghasilan sebatian ini yang tinggi dalam industri telah menimbulkan kebimbangan terhadap kesan kesihatan disebabkan oleh sifat ketahanan dan potensi toksiknya. Sifat bioakumulatif sebatian ini telah membawa kepada pengumpulannya di dalam matriks alam sekitar, sampel manusia serta sumber dan produk makanan. Pengambilan makanan dikesan sebagai sumber pendedahan yang penting bagi PFOS dan PFOA terhadap manusia. Justeru, objektif utama penyelidikan ini adalah untuk menilai tahap kepekatan PFOS dan PFOA di dalam telur poltri yang dihasilkan secara komersil dan ternakan di rumah, di Malaysia. Sebanyak 47 sampel kuning telur yang terdiri daripada telur ayam (n=40), telur itik (n=3) dan telur burung puyuh (n=4) telah dikumpulkan daripada sumber yang berbeza dalam Malaysia. Sampel-sampel ini diekstrak melalui teknik pemendapan protein ringkas menggunakan asetonitril. Kaedah analisis telah dibangunkan menggunakan LC-MS/MS dan disahkan menggunakan garis panduan FDA 'Bioanalytical Method Validation' untuk memastikan kualiti dan kebolehpercayaan kaedah tersebut. Analisis yang dijalankan terhadap sampel yang disaring menunjukkan kehadiran PFOS dalam enam sampel dengan julat kepekatan daripada 0.5 hingga 1.01 ng g^{-1}. Daripada jumlah tersebut,

lima sampel adalah telur ayam daripada sumber ternakan di rumah dan satu sampel daripada telur burung puyuh. Tahap PFOA di dalam kesemua sampel adalah di bawah had kuantitasi (<0.1 ng g^{-1}). PFOS dan PFOA tidak dapat dikesan secara kuantitatif di dalam semua telur ayam daripada sumber komersil. Ini mendedahkan bahawa pencemaran PFC di dalam telur poltri, kebanyakkannya berpunca daripada sifat haiwan ternakan yang bebas berkeliaran dan terdedah secara langsung dengan sumber pencemaran di dalam tanah dan makanan. Kesimpulannya, kaedah analisis yang pantas dan teguh bagi menganalisa PFOS dan PFOA di dalam kuning telur menggunakan LC-MS/MS telah berjaya dihasilkan. Analisa terhadap sampel menunjukkan kehadiran bahan tercemar yang meluas dalam alam sekitar. Kajian lanjutan mengenai penilaian risiko terhadap pendedahan PFC melalui diet adalah disarankan.

Kata kunci: Peiflurooktana sulfonat, asid peiflurooktanoik, telur poltri, kromatografi cecair spektrometer jisim tandem.

LEVELS OF PERFLUOROOCTANE SULFONATE (PFOS) AND PERFLUOROOCTANOIC ACID (PFOA) IN HOME AND COMMERCIALLY PRODUCED POULTRY EGGS IN MALAYSIA

ABSTRACT

Perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are the two major perfluorinated compounds (PFCs), widely used in industrial and consumer products pertaining to their unique water and grease repellent properties. The large industrial manufacture of these compounds had raised substantial concern on health effects, due to their persistence nature and potential toxicity. The bioaccumulative properties resulted in their accumulation in environmental matrices, human samples, as well as in food sources and food products. In human, food consumption was reportedly to be a significant source of exposure for both PFOS and PFOA. Hence, the primary objective of this study was to determine the level of PFOS and PFOA in the yolk of poultry eggs in Malaysia. A total of 47 egg yolk samples, consisting of chicken eggs (n=40), duck eggs (n=3) and quail eggs (n=4) were collected from various sources of areas in Malaysia. These samples are extracted by simple protein precipitation technique using acetonitrile. The analytical method was developed using LC-MS/MS and validated based on FDA's Bioanalytical Method Validation to ensure the quality and reliability of the method. The analysis of the samples revealed that PFOS was quantitatively detected in six samples with the concentration range between 0.5 to 1.01 ng g^{-1}. Among these, five samples are from home produced chicken eggs, and one sample was from a quail egg. The level of PFOA in all the samples were below the quantifiable limit (<0.1 ng g^{-1}). In chicken eggs, neither PFOS nor PFOA

were quantitatively detected in all the commercially produced source. This reveals that the contamination of PFCs in poultry eggs are mostly attributed to the nature of free foraging animals which have direct contact with the contaminants in soils and feeds. In conclusion, a fast and robust analytical method for analyzing PFOS and PFOA in egg yolk samples using LC-MS/MS was successfully developed and validated. The presence of these emerging contaminants in this study, indicated the widespread pollution in the environment. Further study on risk assessment towards dietary exposure of PFCs are suggested.

Keywords: Perfluorooctane sulfonate, perfluorooctanoic acid, poultry eggs, liquid chromatography tandem mass spectrometry.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Perfluorinated compounds (PFCs) are anthropogenic organic chemicals which are widely used as surfactants and surface protectors in many products, pertaining to its unique characteristics of grease, stain and water repellent (Xiao *et al.*, 2015). The examples of PFCs' use include as basic materials in automobile, aviation, chemical industries, textiles, electronic, as well as in semi conductors. These chemicals have high chemical and biological stability, mainly attributed by the chemical structure, displayed by the strength of bond between carbon and fluorine atoms (Zafeiraki *et al.*, 2015). Hence, PFCs are found ubiquitously in the environment, and become bioaccumulated in the food chain (Pan *et al.*, 2014).

The two most commonly discussed PFCs are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), which are globally identified, and recently had raised international concern. Both PFOS and PFOA are long chain perfluoroalkyl substances (eight carbon chain) with the following chemical formulas: $C_8F_{17}SO^-_3$ and $C_8F_{15}COO^-$ (Suja, Pramanik & Zain, 2009). Unlike other classical lipophilic persistent pollutants such as dioxin and polychlorinated biphenyls, both PFOS and PFOA do not typically accumulate in lipids, but rather in body compartments with high protein content (Jones *et al.*, 2003). Toxicology studies in animals and biomonitoring data from occupationally exposed workers (in PFCs industries), had shown the potential of these compounds to cause health implications associated with liver toxicity, immunological and endocrine disruption, development toxicity, as well as cancer (Kudo & Kawashima, 2003).

The aquatic ecosystem had been recognized as a significant medium for PFCs transport. The released of these compounds into the aquatic environment are reported to be through point source (industrial and sewage treatment plant) and non-point source (surface run-offs and atmospheric discharges), which subsequently lead to their bioaccumulation in the food web and thus, possess potential health risk to both human and animals (Suja *et al.*, 2009). A study conducted by So *et al.*, (2004), have reported a substantial pollution of PFOS and PFOA in the surface water of East Asia, particularly in Hong Kong, China and Korea. Due to the increase in concentration of PFOS and PFOA in aquatic environment, the toxicity and ecological risks to aquatic biota had also increased (Houde *et al.*, 2011).

The main pathway for human exposure to PFCs would be from the dietary intake, sourced from contaminated food and water (Sunderland *et al.*, 2019). The intake of fish and marine mammals were reportedly the main contributor to the dietary exposure to PFCs, apart from their presence in various other food sources (Christensen *et al.*, 2017). In the recent years, presence of PFCs in chicken eggs was reported (Wang *et al.*, 2008; Zafeiraki *et al.*, 2015). Chicken eggs are identified as the common source of protein intake in human diet. Some of these eggs are collected from chickens that are reared non-commercially, free-foraging, and mainly feeds by pecking worms or small insects from the soil. These chickens, which are exposed to the external environment, may have their products (for example eggs) become contaminated with pollutants, such as PFCs.

To date, not much of information is available concerning human exposure to PFCs from dietary intake in Malaysia. Therefore, in this study, we aim to investigate the PFCs contamination in poultry eggs (chicken, duck and quail) in Malaysia, and

compare the level of contamination between commercially produced eggs, with the eggs collected from home-produced source.

1.2 Research statement

In this dissertation research project, the analysis of two perfluorinated compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in poultry eggs were subjected for the study. Both of these PFCs were detected in chicken-based products from previous study in China, Netherland and Greece (Wang *et al.*, 2008; Zafeiraki *et al.*, 2015). According to the Department of Statistics Malaysia, the per capita consumption (PCC) of poultry products in Malaysia (2016), in particular of chicken and duck eggs, was found to be 21.3 kg/year (DOSM, 2017). Eggs, have been recognized as one of the preferred food by Malaysian, as these are cheaper source of proteins, compared to others. The sources of some of these eggs are from free-foraging poultries, that are exposed to external environment, and hence their products are potentially contaminated with pollutants such as the perfluorinated compounds. In Malaysia, study of PFCs in food samples are scarce. Therefore, there is a need to develop a sensitive and precise analytical method, to detect the presence of these compounds in poultry eggs in Malaysia.

1.3 Study objectives

1.3.1 General objective

To determine the level of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in the yolk of poultry eggs (chicken, duck and quail) in Malaysia.

1.3.2 Specific objectives

i. To develop and validate a fast and robust analytical method for determining level of PFOS and PFOA in chicken, duck and quail eggs using LC-MS/MS.
ii. To determine and compare the level of PFOS and PFOA between chicken eggs collected from home produced and commercially produced chickens.
iii. To compare the level of PFOS and PFOA in chicken, duck and quail eggs (across species).

CHAPTER 2

LITERATURE REVIEW

2.1 Perfluorinated compounds

Perfluorinated compounds (PFCs), which are also commonly referred to as perfluoroalkyl and polyfluoroalkyl substances (PFAS), are a group of anthropogenic chemicals which are widely used in industrial and consumer applications. These complex heterogenous group of compounds, have been in use for more than 60 years, since 1950s (EPA, 2017), as surface protecting agents in cookwares, upholstery and water-resistant clothing. Structurally, PFCs are organic compounds consisting of a carbon backbone surrounded by fluorine, which contributes to their resistance to heat, acid or other forces that lead to chemical degradation (Posner, 2012). The stable fluorine-carbon bonds, in addition to the hydrophobic and lipophobic characteristics, lead to their enduring properties and usefulness as surfactants and polymers (Kissa, 2001).

The history of PFC production are mostly tailed back to the year 1949, when perfluorooctane sulfonate and its related substances was globally manufactured by the 3M™ company. Between 1970 to 2002, it was reported that the total cumulative production was estimated to be nearly 96, 000 tonnes (Paul, Jones & Sweetman, 2009). The 3M™ plant in Minnesota (USA) was the main producer, and became the most studied for contamination and pollution. It was until 2002, the company finally decided to voluntarily phase out the production of PFOA and PFOS-related compounds (Oliaei *et al.*, 2013).

The production of perfluorinated compounds involves several different processes. The two major processes electrochemical fluorination (ECF) and

telomerization (Buck *et al.*, 2011). Electrochemical fluorination is a process that produce mixture of isomers and homologues. This process generates numerous by-products, including branched and linear isomers of even and odd numbered chain lengths. ECF was used by 3M™ company for the perfluorination of n-octanoyl halide to produce perfluorooctylsulfonyl fluoride, which are used as a starting material for perfluorinated sulfonamide products (Benskin, De Silva & Martin, 2010).

In contrast to electrochemical fluorination, telomerization is a polymerization process that yields an isomerically pure products, retaining the structure of the starting material (Benskin, Bataineh & Martin, 2007). In telomerization, a perfluoroalkyl iodide is reacted with tetrafluoroethylene, resulting in mixture of perfluoroalkyl iodides with longer perfluorinated chains (formed in the 1st step of telomerization) and fluorotelomer iodides (formed in the 2nd step of telomerization). These intermediates are further reacted to form a wide variety of 'fluorotelomer-based' products (Buck *et al.*, 2011). The most common are fluorotelomer alcohols (FTOHs), which are utilized as raw materials in the synthesis of fluorotelomer-based surfactants and polymers (Kannan, 2011). They are mainly used to produce waterproof textiles and additive of paper products (Posner, 2012). Degradation of FTOHs leads to the yield of perfluoroalkyl carboxylic acids (PFCAs).

Collectively, there are 42 families and subfamilies of perfluorinated chemicals, consisting the total of several hundred compounds. These compounds are usually differentiated based on the length of the carbon chain, presenting as either 'long-chain' or 'short chain' perfluorinated compounds. Between these two, the long chain are more of concern, due to their bioaccumulative properties. The two major classes of long chain PFCs are perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic

acids (Buck *et al.*, 2011). Perfluoroalkyl carboxylic acids (PFCA) are compounds which consist of eight carbons or greater, with a terminal carboxylic acid functional group. Some examples of PFCA are such as perfluorooctanoic acid (PFOA), perfluorononanoate (PFNA) and perfluorobutanoate (PFBA). Perfluoroalkyl sulfonic acids (PFSA) are compounds which consist of six carbons or greater, with a terminal sulfonic acid functional group. The examples of some PFSA include perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorobutane sulfonate (PFBS) (Benskin *et al.*, 2010). Between PFCA and PFSA, the latter are shown to be more bioaccumulative, compared to PFCAs presenting with the same fluorinated carbon chain length (Conder *et al.*, 2008). The general structures of perfluoroalkyl carboxylic acids and perfluoroalkyl sulfonic acids are illustrated in Figure 2.1.

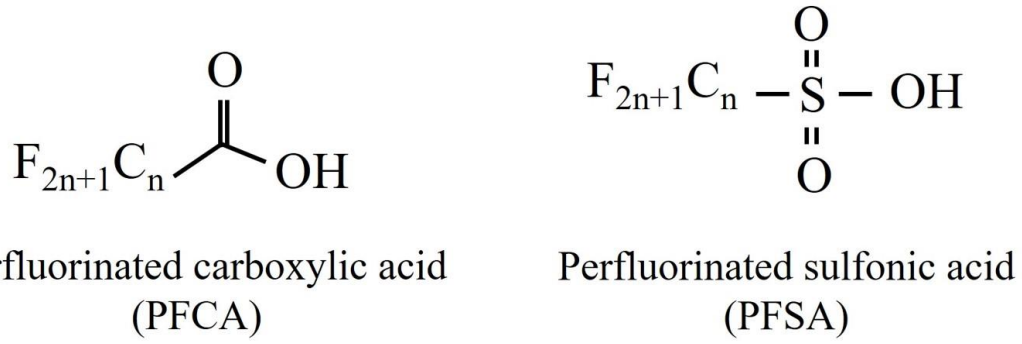

Figure 2.1 General structures of perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkyl sulfonic acids (PFSAs) (Adapted from Rayne & Forest (2009)).

Among all members of the PFCA and PFSA subclasses, the two most important and widely discussed are perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA). The numerous use of the PFC-containing products have contribute to the widespread presence of PFOS and PFOA in the environment.

Previous studies have described the presence of PFOS and PFOA in water, soil, indoor air, house dust, food products, fish, avian eggs, as well as in human blood serum (Series, 2010; Malinsky *et al.*, 2011; Ericson Jogsten *et al.*, 2012; Vicente *et al.*, 2012; Xiao *et al.*, 2015; Zeng *et al.*, 2015). Due to their toxicity and bioaccumulative characteristics, both of these compounds have raised health concern, especially PFOS, which satisfy the defining criterias of persistent organic pollutant (POPs) (Corsini *et al.*, 2014).

2.1.1 Perfluorooctane sulfonate

Perfluorooctane sulfonate (PFOS) is the predominant PFSA containing of eight carbon, fully-fluorinated backbone, with an added sulfonate group. The chemical structure of PFOS ($C_8HF_{17}O_3S$) is displayed in Figure 2.2.

PFOS and its salts are fluorinated organic compounds, belonging to the PFSA class. The production of PFOS begin with electrochemical fluorination (ECF) of n-octanesulfonyl fluoride, which yield the parent compound, perfluorooctane sulfonyl fluoride (PSOSF). Subsequently, base-catalyzed hydrolysis of PSOSF yields PFOS (Benskin *et al.*, 2010). Physicochemical properties of PFOS are describe as in Table 2.1.

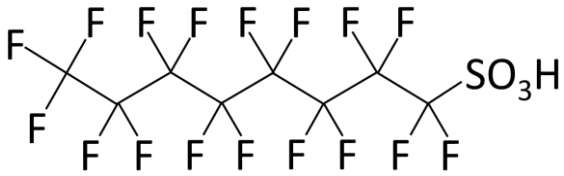

Figure 2.2 Chemical structure of PFOS (Adapted from from Smith *et al.*, (2016)).

Table 2.1 Physicochemical properties of PFOS (Adapted from EPA 2017 and OECD 2002).

Physicochemical properties	PFOS
Molecular weight (g/mol)	500
Boiling point (°C)	Not calculable
Melting point (°C)	258-260
Vapor pressure at 25°C (mm Hg)	0.002
Water solubility at 25°C (mg/L)	680

PFOS is used in the form of either the undissociated sulfonic acid or one of its sulfonate salts (Smith *et al.*, 2016). Due to its surface-active properties, PFOS and it's related substances are used in a variety of applications. One of the example is the use as surface treatment, providing soil, oil and water resistance to textile and home furnishing. PFOS is also used in paper treatment applications, and as performance chemicals, such as in fire fighting foams and electronic chemicals (OECD, 2002).

PFOS was included in Annex B of the Stockholm Convention in May 2009, as it fulfills the characteristics and definition of persistent organic pollutants. Since then, the manufacture and usage of PFOS and related chemicals has been restricted in signatory countries to the Convention, with exception to certain applications which cannot be replaced by other chemicals than PFOS (Smith *et al.*, 2016).

2.1.2 Perfluorooctanoic acid

Among the members of PFCAs, perfluorooctanoic acid (PFOA) is the most commonly encountered compound in the environment. The structure of PFOA ($C_8HF_{15}O_2$) consists a chain of seven perfluorinated carbon atoms, and a carboxyl head group (Figure 2.3).

The free acid of PFOA is expected to dissociate completely in water, leaving the anionic carboxylate in the water and the perfluoroalkyl chain on the surface (European Food Safety Authority, 2008). PFOA is primarily used as polymerization aid, in the manufacture of various fluoropolymers, such as polytetrafluoroethylene (PTFE). These polymers, such as Teflon are used to coat cookware, and as surfactant in soap (Fujii *et al.*, 2007).

PFOA, similarly as PFOS, has raised concern as these chemicals have relatively low vapour pressure, high solubility in water, and have high resistance towards environmental degradation. The European Commission has published regulation to restrict the manufacture, marketing, and use of PFOA, it's salts and related substances (The European Commission, 2017). Physicochemical properties of PFOA are describe as in Table 2.2.

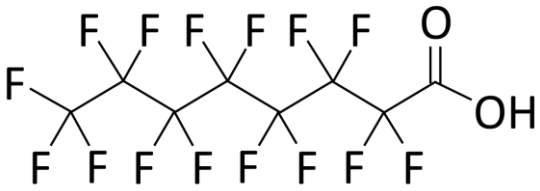

Figure 2.3 Chemical structure of PFOA (Adapted from Smith *et al.*, (2016)).

Table 2.2 Physicochemical properties of PFOA (Adapted from EPA 2017).

Physicochemical properties	PFOA
Molecular weight (g/mol)	414
Boiling point (°C)	192
Melting point (°C)	54
Vapor pressure at 25°C (mm Hg)	0.525
Water solubility at 25°C (mg/L)	9.5×10^3

2.2 Environmental fate of PFOS and PFOA

The detection of PFOS and PFOA in tissues of various species of wildlife has sparked initial concern and interest on research of PFCs in the environment (Giesy & Kannan, 2001). The widespread distribution of these perfluorinated compounds in the global environment are attributed by their high solubility in water, low to moderate soil and sediment sorption, and resistance to biological and chemical degradation (Smith *et al.*, 2016). Aquatic environments are recognized to be the major compartment for PFCs in the environment, and previous studies have perceived ocean as the ultimate reservoir for PFCs (Yamashita *et al.*, 2008). Therefore, in order to evaluate the fate of PFOS and PFOA in the environment, knowledge of the transport, bioaccumulation, biomagnification and degradation pathways are important.

2.2.1 Transport

Water bodies serve as an important medium in the global transport of perfluorinated compounds. Transport of PFCs by the ocean water occurs as the combination of PFCs discharge to surface waters, atmospheric loading, discharge of precursors to surface water and transformation of precursors to PFCs (Prevedouros *et al.*, 2006). Due to their high water solubility and persistence, PFOS and PFOA undergo minimal degradation (Kannan, 2011). Furthermore, the mobility of perfluorinated compounds in water are partly influenced by the degree to which the PFCs sorb to sediment and soil during transportation (Smith *et al.*, 2016).

The transport of PFCs also occurs through atmospheric medium. It was reported that the movement of PFCs into atmosphere may occur through volatilization

from water bodies and soils, and these compounds can be subjected to long distance atmospheric transport (Li *et al.*, 2011). A study conducted by Liu *et al.*, (2015), demonstrated the detection of PFCs in the atmosphere of Shenzhen, China with PFOS and PFOA as the major components. In addition, the study also found that the source of PFCs in the Shenzhen atmosphere was due to long-distance transport of pollutants from the southeastern coastal area of the region.

2.2.2 Bioaccumulation

Various field study have described biomagnification of PFCs in food webs, particularly involving the long chain forms. It was reported that there is a direct relationship between the length of carbon chain and the bioaccumulation of PFCs. A study conducted on rainbow trout (*Oncorhyncus mykiss*) by Martin *et al.,* (2003) revealed that both bioaccumulation factors (BAFs) and bioconcentration factors (BCFs) increased, as the length of perfluoroalkyl chain increases. The BCFs and BAFs of the perfluoroalkyl sulfonates are found to be greater than the corresponding carboxylates of equal perfluoroalkyl chain length. This indicated that apart from the chain length, the acid functional group are also accounted for the bioaccumulation feature.

The assessment of biomagnification can be done through determination of biomagnification factor (BMF) or trophic magnification factor (TMF). BMF value of more than 1 (BMF>1) indicates the biomagnification of the substance between prey and predator. The TMF provides evidence on the chemical's ability to biomagnify in food webs (TMF>1). A review by Houde *et al.,* (2011) on several marine and

freshwater food webs studies, showed that most of the BMFs and TMFs for PFCs were reportedly greater than 1.

More studies are progressing towards understanding the fundamental criterias of perfluorinated compounds bioaccumulation. A study using novel-protein binding model of PFCs in fish reported that protein interactions are the key to predict the tissue-specific PFCs bioconcentration (Ng & Hungerbühler, 2013).

2.2.3 Degradation

Both PFOS and PFOA are known to be highly persistent in the environment. However, at certain point, these compound can undergo degradation by chemical methods, such as advanced oxidation and photodegradation processes. In advanced oxidation method, the use of subcritical water as a strong oxidant, catalyzed with Fe, was found to be capable of degrading PFOS completely (Hori *et al.*, 2006). A study by Taniyasu *et al.*, (2013), showed that long chain PFCs can be degraded to short chain PFCS when these are subjected to photodegradation under strong solar radiation, though the process is energy intensive and occurs at slow rate. Apart from chemical degradation, PFCs may also be subjected towards biodegradation. Biodegradation of PFOS and PFOA in sludge of wastewater treatment plant were studied in aerobic and anaerobic reactors (Meesters & Schröder, 2004). The result revealed that both PFOS and PFOA surfactants could be eliminated successfully under anaerobic treatment, but no biodegradation was observed under aerobic treatment.

2.3 Toxicokinetics of perfluorinated compounds

2.3.1 Absorption/uptake

Human exposure towards of PFCs mainly occurs via ingestion of contaminated foods or water (dietary uptake). The source of contamination in foods may come from the production processes and/or contact with PFCs-coated cookwares (European Food Safety Authority, 2008). Both PFOS and PFOA are readily absorbed by the gastrointestinal tract, following oral exposure (OECD, 2009). Apart from oral exposure, the uptake of PFOS and PFOS may also occur due to inhalation or dermal contact with dust or aerosols containing PFCs. However, the dermal exposure occurs at lesser significance, compared to ingestion and inhalation (Kudo & Kawashima, 2003).

2.3.2 Distribution

Unlike most other persistent organic pollutants (PCDD/Fs, PCBs) perfluorinated compounds such as PFOS and PFOA have low affinity to lipids, very water soluble, and bind preferentially to protein. The distribution and accumulation of PFOS and PFOA occurs mainly in plasma, liver, and kidney (Stahl, Mattern & Brunn, 2011). It was suggested that in PFOS molecule, the physicochemical structure contribute to the interactions of either the sulfonic acid group, or the hydrophobic alkyl chain with serum proteins. PFOS principally binds to serum albumin, and it was demonstrated that the binding occurs strongly at 1:1 stoichiometric ratio (Jones *et al.*, 2003). The chain length and the acid head group of the PFCs have a significant

influence on its's preference for binding sites and binding affinity. These chemicals binds to serum albumin at the similar binding site and similar affinity, as fatty acids (Chen & Guo, 2009).

2.3.3 Metabolism

As far as concern, both PFOS and PFOA are recalcitrant towards metabolism in mammals. Generally, all PFCs resist catabolism and phase II conjugation, and they are poorly excreted in humans (Stahl *et al.*, 2011). The metabolism of perfluorinated compounds are only reported to occur in the precursors, such as FTOH. Studies have shown the formation of PFCAs occurring from metabolism of telomer-based precursors (Martin, Mabury & O'Brien, 2005; Vestergren *et al.*, 2008). In this case, the alcohol groups undergoes oxidation to form fluorotelomer aldehyde, followed by oxidation to saturated fluorotelomer compounds, such as FTCA (fluorotelomer saturated carboxylate). It was demonstrated that FTOHs can be metabolized to perfluorinated carboxylic acids of various chain lengths, suggesting an explanation for the presence of long chain PFCs in human blood (Martin *et al.*, 2005).

In addition, both PFOS and PFOA are shown to have the capability in crossing the placental barrier. A pilot study conducted by Midasch *et al.*, (2007), indicated a decrease of PFOS concentration from maternal to cord plasma by a factor of 0.41 to 0.80, but on contrary, the PFOA concentration were higher in the placenta compared to the maternal plasma (ratio of cord plasma: maternal concentration range from 0.91 to 1.95). Nevertheless, the outcome showed that PFOS and PFOA can cross the placental barrier, and may lead to harmful consequences towards the neonatal

development. PFOS and PFOA have also been detected in human breast milk, at low concentration (Mondal *et al.*, 2014).

2.3.4 Excretion

Due to the carbon-fluorine stability and high electronegativity of the perfluorinated alkyl chains, both PFOS and PFOA are excreted in urine and faeces, without undergoing biotransformation. Urinary excretion served as the important pathway in the elimination of PFCs from the body, as shown in previous studies (Butenhoff *et al.*, 2004; Cui *et al.*, 2010). Urinary excretion represents a fraction of the systemically absorbed oral dose of toxicants excreted through urine, while the fecal excretion represents systemically absorbed toxicants present in the digestive tract, as well as portion of unabsorbed toxicants (Cui *et al.*, 2010). A study in elimination rates of PFOA in male and female cynomolgus monkeys after oral and intravenous dosing showed urinary elimination half life of approximately 20 to 30 days (Butenhoff *et al.*, 2004). Seacat *et al.*, (2002), reported a half-life of approximately 200 days for PFOS in male and female cynomolgus monkeys following daily oral dosing over six months.

2.4 Toxicity of PFOS and PFOA

Due to their persistent nature, accumulation of PFCs in animals and human can lead to potential health impairment. The presence of PFCs in human sample was suspected in the late 1960s by Taves (1968), when he discovered fluoride in blood samples that are partially bound to organic compounds of unknown structure. The subsequent studies in 1970s reported higher than normal organic fluorine levels in the blood of fluorochemicals industrial workers, indicating the effects of exposure towards perfluorochemicals (Ubel, Sorenson & Roach, 1980). Apparent studies on perfluorinated compounds begun in the 2000s, as PFCs were found widely distributed in the environment and detected in human blood samples (Lindstrom *et al.*, 2011).

Laboratory animal studies and epidemiological research on human population had demonstrated affiliation between the exposure to certain PFCs to various adverse health effects (Sunderland *et al.*, 2019).

2.4.1 Body and organ weight changes

A study on subchronic toxicity of PFOS potassium salt in cynomolgus monkeys revealed the significant loss of initial body weight in the monkeys given oral dose of 0.75 mg/kg/day, for 182 days. Additionally, there was also a significant increase of liver-to-body weight ratios in both female and male cynomolgus monkeys (Seacat *et al.*, 2002). A study by Cui *et al.*, (2009) showed a sharp loss of body weight in the Sprague-Dawley rats given the high exposure of PFOS at 20 mg/kg. Nonetheless, the results on body weight effects of PFCs differs between animals and human. In human, Liu *et al.*, (2018) discovered the effect of PFCs causing interference

with human weight regulation, which may lead to obesity. During the study, the researchers found that the greater PFCs concentration in the blood, the greater weight was gained back after the initial period of weight loss. The findings were also supplemented with a slower regression of resting metabolic rate (RMR), which is defined as the amount of energy burned when the body was at rest.

2.4.2 Hepatic toxicity

Studies on laboratory animals had revealed the potential of hepatic toxicity, due to exposure towards PFCs. Histopathological observation on exposure towards PFOS and PFOA in Sprague-Dawley rats revealed cytoplasmic vacuolation, focal or flakelike necrosis and hepatocellular hypertrophy seen in liver of each treated group (Cui *et al.*, 2009). In the study, exposure at high dose was associated with liver focal haemorrhage, erythrocytic transudation, and focal hepatocytic degeneration accompanied by inflammatory cellular infiltration.

Liver toxicity described by hepatocellular adenomas, hepatocellular hypertrophy and bile duct hyperplasia were observed in two mice strains (CD-1 and 129/Sv) exposed to gestational PFOA exposure, in the period of 18 months (Filgo *et al.*, 2015). The proposed mechanism of action towards PFOA-induced hepatic toxicity in rodents include the PPAR-α (peroxisome proliferator activated receptor-alpha) pathway and mitochondrial disruption (Klaunig *et al.*, 2012; Filgo *et al.*, 2015). However, these mechanism are yet completely characterized, and may be implausible towards toxicity in human liver.

2.4.3 Serum lipid level

Studies on occupationally exposed workers indicated positive association between the serum level of PFOS and PFOA, with serum lipid level, such as cholesterol (Olsen *et al.*, 2000, 2003; Costa, Sartori & Consonni, 2009). In addition, Sakr *et al.*, (2007), through a cross sectional study on DuPont workers, observed a modest but statistically significant positive correlation between serum PFOA and total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), after adjustment for potential cofounders. In general population, the manifestation of dyslipidemia (abnormal blood lipids), was shown to be associated with environmental exposure towards PFCs (Geiger *et al.*, 2014). Dyslipidemia is strongly related to increased risk of cardiovascular disorder, hence, this prompt the likelihood that exposure towards PFCs may lead to cardiovascular complications.

In contrast, the results on animal studies showed an opposing association between PFCs exposure and serum lipid. A study in mice exposed with 5 mg/kg PFOS indicated reduced serum cholesterol level, high density lipoprotein (HDL) and low density lipoprotein (LDL), but an elevated serum triglyceride level. The release of ventral fat store and elevated serum albumin are proposed to be the factors that bring to the increase in the triglyceride level (Wang *et al.*, 2014). These opposed findings between human and animal studies were suggestively attributed to the dissimilar response of PPAR-α mechanism between the two species. PPAR-α showed an important role in lipid homeostasis, in addition to other physiological process regulations, such as wound healing, reproduction and carcinogenesis (Corsini *et al.*, 2011). In human liver, the PPAR-α response were either absent, or much reduced compared to the rodents (Klaunig *et al.*, 2003).

2.4.4 Immunotoxicity

Both PFOS and PFOA have been reported to prompt immunomodulations in laboratory animal models. The alteration of immunity are mostly associated with the suppression in the antibody response, as demonstrated by Peden-Adams *et al.*, (2008). In the study, humoral immune effect was described as the most sensitive immune endpoint, and the suppression of antibody production suggested that cellular or molecular target for the humoral immunity suppression are attributed to the alterations in B-cells or antigen presenting cells (APCs). DeWitt *et al.*, (2008) discovered that exposure towards PFOA, lead to suppression of IgM antibodies and reduced mean spleen and thymus weight in adult female mice. In human, data on immunotoxicity are limited, however, some studies in human suggested that PFCs exposure exhibit immunosuppressive effects, especially in early life (Fei *et al.*, 2010; Granum *et al.*, 2013).

2.4.5 Other health effects

Evidence on cancer risks following exposure to PFCs have been shown by *in vitro* and animal studies. The long chain PFCs are potential carcinogens, which may display pathological consequences through generation of oxidative stress. An *in vitro* study using human hepatoma cell line, HepG2, indicated the increase of reactive oxygen species (ROS) generation in culture treated with several PFCs. The uncontrolled increase of ROS may lead to DNA damage, which may suggest potential cytotoxicity and genotoxicity of PFCs in human (Wielsøe *et al.*, 2015). Apart from that, serum level of PFCs are also associated with reproductive dysfunction, involving

infertility (Fei *et al.*, 2009) and poor semen quality (Vested *et al.*, 2013) in human population.

2.5 Regulation enforcement on perfluorinated compounds

The environmental and health concerns of perfluorinated compounds, especially PFOS and PFOA had drawn substantial scientific and public attention. PFOS and its related compounds, was considered to have met the criteria of a persistent organic pollutants, and therefore was listed in the Annex B of Stockholm Convention, 2009 (Wang *et al.*, 2009). This decision results in prohibition or restriction on the production and use of chemicals with distinguish persistent organic pollutants criteria (persistence, bioaccumulative, toxic, and long-range transportable nature). As PFOS was listed in Annex B, their use and production are still permitted for limited specific purposes, such as for medical devices, fire-fighting foam, semi-conductors, photo processing, amongst a few others (UNEP, 2006).

Subsequently, this decision has led many countries around the globe to execute investigations and tried to establish countermeasure in regards to the issue. European Union Directive 2006/122/EC announced restrictions on the marketing and use of PFOS, and stated that the Commission should monitor the risk assessment on PFOA, which posed similar risk profile to PFOS (European Union, 2006).

U.S. Environmental Protection Agency (USEPA) has established health advisory levels at 70 parts per trillion for PFOA and PFOS in drinking water system. This health advisory levels provides a margin of protection for the American populations, from the adverse health implications due to exposure of PFOS and PFOA in drinking water. At present, EPA is evaluating these two major PFCs as drinking water contaminants, in accordance to the Safe Drinking Water Act (SDWA). In

addition, EPA proposed a Significant New Use Rule (SNUR) under Toxic Substance Control Acts, towards PFOA, to ensure that they will have opportunity to review the use, and regulate any proposed new activities (USEPA, 2016). USEPA invited major fluoropolymer and fluorotelomer manufacturers to join in a global stewardship program in 2006. As a result, eight major PFOA manufacturers (Arkema, Ashahi, Ciba, Clariant, Daikin, 3M/Dyneon, DuPont and Solvay Solexis) enrolled in the stewardship, with the aim in achieving the goal of 95 percent reduction in the emission of PFOA, its precursor and related homologues by the end of 2010 (based on the year 2000 baseline). These participants are also committed to work towards 100 percent elimination of these chemicals from emission and products by the end of 2015 (USEPA, 2006).

In Canada, PFOS was included in The Regulations Amending the Prohibition of Certain Toxic Substances Regulations 2012, which came into force on 2016. The regulations prohibit the import, manufacture, use, sale and offer for sale of PFOS and products containing PFOS, with a limited number of exemptions (Government of Canada, 2016). This regulation also enlisted PFOA and its related compounds, prohibiting the compounds and products containing them, unless present in manufactured items.

2.6 PFOS and PFOA in foods

Apart from occupational exposure, human are exposed to the potential hazards of perfluorinated compounds through consumption of contaminated food and water sources. The major exposure was recognized to be from oral route, mainly from dietary intake (Fromme *et al.*, 2009). These are much of a concern, considering the potential of perfluorinated compounds to bioaccumulate in the food chain. In 2008, European Food Safety Authority had established tolerable daily intakes (TDI) at 150 ng/kg for PFOS and 1500 ng/kg for PFOA (European Food Safety Authority, 2008). The study findings of PFOA and PFOS in foods are tabulated in Table 2.3.

2.6.1 Contamination in fish

Fish have been numerously recognized as an important dietary source of perfluorinated compounds in consumers. Various studies have demonstrated that both PFOS and PFOA are able to bioaccumulate in fish (Taniyasu *et al.*, 2003; Pan *et al.*, 2014; Squadrone *et al.*, 2014). It was discovered that PFOS was the dominant PFCs in fish samples, which occur at concentration ranging from <1 ng/g (w/w) to >100 ng/g (w/w), depending on the locations. The highest PFOS concentration in fish, was discovered in samples collected from the Mississippi River, down stream of the 3M plant. During the survey, they found that the PFOS concentration in the blood sample of white bass was 29,600 ng/g, and in the liver of a smallmouth bass, PFOS concentration was up to 6,350 ng/g (Oliaei *et al.*, 2013). It can be deduced from these findings that the concentration of PFOS in fish were strongly correlated to the concentration of PFOS in the water.

Tissue distribution of perfluorinated compounds in fish, indicated high concentration in liver, compared to the muscles, suggesting high binding affinity of PFCs to liver fatty acid-binding protein. In addition, there was an increasing trend of certain PFCs concentration with increase in the fish's length and weight (Pan *et al.*, 2014).

2.6.2 Meat products

Animal meat, particularly of ruminants, was reportedly to contain substantial concentration of perfluorinated compounds, after fish. The environmental exposure of agricultural animals to feed, water or air, results in the presence of PFCs in meat and the related products. Tittlemier *et al.*, (2007) reported relatively high level of PFCs in meat products, compared to all the food products analyzed in the study. Among all the PFCs screened, PFOS and PFOA were detected frequently in the meat, which are similar to the findings from other studies of farm animals in Beijing (J. M. Wang *et al.*, 2010b), Spain (Martí-Cid *et al.*, 2008) and USA (Lupton *et al.*, 2014).

2.6.3 Dairy products

Several studies have been made regarding bioaccumulation of perfluorinated compounds in dairy products (D B Clarke *et al.*, 2010; Haug *et al.*, 2010; J. M. Wang *et al.*, 2010a; Noorlander *et al.*, 2011; Kowalczyk *et al.*, 2013; Barbarossa *et al.*, 2014). The presence of perfluorinated compounds in agricultural products such as milk, indicated the transfer of contaminants from the feed, to the ruminant's tissue and excretions. A study by Kowalczyk *et al.*, (2013), revealed a high concentration of PFOS (24.2 ± 9.0 μg/L), compared to other PFCs, in the studied cow's milk samples.