Methodologies and Results Concerning the Determination of Ocs And Pcbs From Fish Meat - *A Mini Review*

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Abstract

In the last decade, studies have been focusing on determinations of persistent organic pollutants (POPs), well known for being involved in human chronic diseases. Various approaches and methodologies are used in monitoring these chemical substances in fish food chain. Therefore, their development, functionality and efficiency are vital for consumers' protection. The aim of this paper is to review recently published analytical techniques for sample preparation in the determination of POP residues such as organochlorine compounds (OCs) and polychlorinated biphenyls (PCBs). Limitations and advantages of sample preparation techniques are discussed and compared so that they can facilitate the decision of future analysts upon adequate protocols in individual laboratories. A list of the most common compounds quantified nowadays is displayed. In addition, results achieved in original papers are used to briefly describe the current situation on different continents, with third world countries undergoing more pollution than the rest of the world.

Keywords: fish, consumers protection, analytical techniques, OCs, PCBs

Introduction

Pesticides involve a variety of products such as insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides, plant growth regulators and others. Each of these products, besides their primary benefits, spread dangerous chemicals in the environment and their toxicity has negative repercussions on air, soil, water and organisms. A group in particular- insecticides, contains organochlorine compounds (OCs) known for their high toxicity, slow degradation and bioaccumulation (Jayaraj et al., 2016). Exposure of the general population to pesticides occurs primarily through eating food and drinking water contaminated with pesticide residues, whereas substantial exposure can also occur in or around the home (Damalas et al., 2011). One way for human individuals to accumulate residues of such compounds and find themselves at health risk is through food ingestion. A good example of how OCs can climb their way into the human body is fish food chain. Fish can come in contact with pesticides from feed made of insecticide-sprayed plants and contaminated water or sediments (Agbohessi et al., 2014). After retaining compound residues in the fat tissue, the transfer can be made

to consumers even if the fish is cooked. Pesticides may induce adverse health effects including cancer, effects on immune or nervous systems (WHO, 2016). Dichlorodiphenyltrichloroethane (DDT) and its breakdown product dichlorophenyl dichloroethylene (p,p′-DDE) affect the developing male and female reproductive organs in human infants and children (Borchers et al., 2010). The effective mechanisms of action of pesticides are genetic damages, epigenetic modifications, endocrine disruption, mitochondrial dysfunction, oxidative stress, endoplasmic reticulum stress and unfolded protein response (UPR), impairment of ubiquitin proteasome system and defective autophagy (Mostafalou and Abdollahi, 2013). Therefore, placing these substances under global control is an important step in protecting the public (Stockholm Convention New POPs, 2005).

Besides OCs, other persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) threaten the environment and hereby the human population. Polychlorinated biphenyls are mixtures of up to 209 individual chlorinated compounds (known as congeners) (ATSDR, 2014). PCBs were used in a wide variety of applications that resulted in release to the environment (Grimm et al., 2015). They were employed in the past as dielectric fluids in power transformers and capacitors, as insulators, coolants, plasticizers in plastic and rubber products, and as hydraulic fluid (Oluoch-Otiego et al., 2016). Even if their mass production and use was stopped or restricted and no natural sources of PCBs are known, traces are still discovered either because of accidental leaks, fires in electrical equipment, past disposal in dumps and leakage from hazardous waste sites or because fires in transformers, capacitors, or other products containing PCBs (ATSDR, 2000). The exposure of humans and animals to large amounts of PCBs generates skin conditions such as acne and rashes, but also liver damage. In other unfortunate cases, animals simply died or developed stomach injuries, immune system suppression, neurological or endocrine problems and impaired reproduction. The U.S. Environmental Protection Agency (U.S. EPA) and International Agency for Research on Cancer (IARC) classify PCBs as probably carcinogenic, respectively carcinogenic to humans (group 1) due to the links found between these compounds and liver cancer (EPA, 2005; Lauby-Secretan et al., 2013).

The analysis of OCs and PCBs in marine biota samples is an essential part of monitoring and managing the risks posed by these compounds in the environment, providing the information required to develop and enforce regulations and to perform ecotoxicological risk assessments (Choi et al., 2016). In the past decade, more analytical methods for accurate identification and quantitative determination of traces of pesticides in marine biota have been developed to improve our understanding of their risk to ecosystems and humans (Andreu & Picó 2012). In order to find the most automated and eco-friendly, but also the fastest and most accurate way to detect and quantify residues of OCs and PCBs, researchers in the field tried a serie of extraction and clean-up protocols. They focused attention on overall performance, aspects such as essentially organic solvent-less approaches, large-volume injection and miniaturization (de Koning et al., 2009). OCs can be determined separately from PCBs, but their physico-chemical characteristics make their determination together also possible. The powerful instrumental technique of gas chromatography coupled to mass spectrometry in the determination allows determinations of OCs in the same time with PCBs, but reliable results can be obtained only by delivering a proper sample preparation. This is a crucial step prior to the analytical determination of these pollutants (Ottonello et al., 2014).

The two important factors in the election of a certain methodology are sample matrix and the structure and properties of the target analytes (Stocka et al., 2011). This paper presents an overview on the current state of the art of modern sample preparation methodologies and analytical instruments for OC and PCB residues analysis in fish, offering current researchers and analysts fundamental information in the build-up of their personal original protocols for POP residue determination in fish. The present paper includes information regarding the most frequent chemical compounds analyzed or found worldwide and some contaminated fish species and sites, with a special emphasis on the works published on this topic in the last five years.

Extraction techniques

The Soxhlet extraction (SE) is a traditional continuous solid-liquid extraction. It is one of the

first extractions used in the determination of OC and PCB residues from fish. The principle of this method is the extraction of fat from solid materials by repeated washing with an organic solvent, under reflux, in a special glassware. Most commonly, in the beginning of the method, the fish sample is grinded with activated sodium sulphate until a fine powder is obtained. Subsequently the mixture is extracted. In this step, authors report the use of different organic solvents, but also different extraction time. Some of the solvents added during this procedure are combinations of n-hexane:dichloromethane, methylene chloride:n-hexane, acetone:n-hexane, acetone:hexane:dichloromethane in different ratios, but also simple solvents such as n-hexane or methylene chloride (Arzi et al., 2011; Kafilzadeh et al., 2012; Shi et al., 2013). The extraction time can range from minutes to hours (Azab et al., 2013; Ezemonye et al., 2015; Huertas et al., 2016) and occasionally there are mentions of overnight extractions. Therefore, SE is a time consuming technique (Andreu and Picó 2012).

Despite reliable results obtained with this technique over time, it is considered now surpassed. Its high reagent consumption is costly and makes it less eco-friendly. The preparation is laborious and the method offers no scope for automation. Another limitation of this method is that it concerns generally one family of compound with similar properties (Lazartigues et al., 2011) in the context in which multiresidue determinations are desired.

The ultrasonic extraction (UE) is a technique which recovers organic analytes from fish or other permeable solid matrix by means of a solvent energized by sound energy at frequencies in excess of those audible to the human ear. The average work instructions start with mixing the sample with anhydrous sodium sulfate and continues with adding the necessary solvents for extraction. Successful results are achieved when merging hexane with acetone in different ratios (Akoto et al., 2016; Ibigbami et al., 2015). After mixing thoroughly by shaking, the process ends with the sonication of the mixture in an ultrasonic bath for 15-20 min and the collection of the supernatant. The extraction is repeated two more times and all of supernatants are combined and concentrated. From time to time lipid components can be coextracted with the supernatant and their adsorption in the injection port or column

of the GC system is inevitable. When a clean-up stage is required, the whole preparation process becomes more laborious previous to the actual quantification. The work can be reduced by coupling UE with other analytical techniques (Seidi and Yamini 2012; Shrivas and Wu 2008).

From the perspective of costs, the ultrasonic water baths are cheap instruments. They are less efficient though than other systems using a cylindrical powerful probe for the sonication of samples (Tadeo et al., 2010). Although the method insures a decreased extraction time and solvent consumption, it employs high power consumption.

Microwave assisted extraction (MAE) uses microwaves to warm the solvents in contact with the solid matrix to extract the contents from the sample solution (Ivanovs and Blumberga 2017). A typical procedure implies mixing the fish sample with anhydrous sodium sulfate powder, loading the extraction cylinders, adding solvents and extracting in a Microwave Accelerated Reaction System. Similar to the case of other extraction methods, the solvents used in MAE can be various: n-hexane:acetone (1:1 v/v), acetonitrile:water (19:1 v/v) (Wang et al., 2010; Wilkowska and Biziuk 2010).

MAE has mainly the same advantages and disadvantages as UE, but an extra particular limitation concerns its moderate reproducibility depending on dispersion of microwaves (Lazartigues et al., 2011).

Accelerated Solvent Extraction (ASE), also known as pressurized liquid extraction (PLE), is a newer technique which has attracted more and more attention in samples pretreatment for residues analysis due to its high-level automation, high extraction efficiency, good selectivity (Zhang et al., 2013). Under elevated temperature and pressure, an extraction solvent can be used above its boiling point but still remains in the liquid state, increasing the kinetics of the extraction process (Mustafa & Turner 2011). As a result, the extraction time and solvent consumption are significantly decreased (Wang et al., 2010). Before extracting, the samples of fish must be treated to remove most or all of the water. There are several ways to achieve this, but one of the most efficient ways is freeze-drying (Botaro et al., 2011; Cioca et al., 2017a, Santhi et al., 2012). Choosing a solvent that matches the chemical profile of the studied analytes and setting convenient parameters such

as temperature, pressure and static cycles are essential. From multiple optimized ASE conditions that are nowadays discussed, the following stand out the most in the extraction of fish fat and POPs: dichloromethane (DCM):hexane (3:7, v/v) as solvent mixture, 60° C-100° C oven temperatures, 1500 psi of pressure and 2-3 static cycles of 5 min (Choi et al., 2016; Ghosh et al., 2011; Shen et al., 2011).

The regular ASE equipment consisting of Dionex™ ASE 200, 300 or 350 supplied by Thermo Scientific Fisher is associated with high cost. This is valid also for spare parts. In contrast with this disadvantage, the machines are easy to use. The sample preparation for extraction is simple and rapid, the high pressure during extraction allows the recovery of thermally labile analytes even at high temperatures and numerous samples can be extracted in one cycle. Extract clean-up is usually necessary due to one major drawback of the method- the presence of a large amount of co-extracted interferences which must be separated from analytes, or chromatographic performance will seriously deteriorate (Shen et al., 2011).

Supercritical fluid extraction (SFE) is a process of analyte separation from fish matrix using supercritical fluids as solvents (Curtean-Bănăduc et al., (2016)). Carbon dioxide (CO_2) is the most popular supercritical fluid because it is inert, non-flammable, non-explosive, cheap, can be easily removed from extracts, can be liquefied and stored in cylinders without problems, and it is considered "green" (Hartmann & Ganzera 2015; Kang et al., 2017). A lower critical temperature and surface tension, better selectivity and like ASE- the ability to shorten extraction time and reduce the amount of organic solvent, place this technique over traditional organic solvent methods (Sapkale et al., 2010). Another practical advantage of SFE is the possibility of performing fractionation during decompression, just by using two or more decompression steps. This cascade depressurization is useful to separate components in the extract (Herrero et al., 2015).

However, an important shortcoming of the technique is that it is unable to extract polar compounds (CO_2 has low polarity). Polar modifiers (also called cosolvents) are commonly added in small amounts in order to overcome this problem (da Silva et al., 2015). The use of few organic solvents, makes it possible to develop less toxic

and pollutant extractions with supercritical fluids (Garcia-Rodriguez et al., 2008).

QuEChERS (quick, easy, cheap, effective, rugged and safe method) is more commonly used in analyzing pesticide residues from fruits and vegetables (Liang et al., 2012). Extractions from other foods with animal origin are being developed more slowly due to matrix complexity. Fish samples are extracted in the presence of a dispersant in a disposable Teflon centrifuge tube. Khorshid et al., 2015 describes the steps of extraction as following: addition of the internal standards to the sample, addition of acetonitrile as solvent, mixture shake for 2 min, addition of Agilent QuEChERs kit for extraction, mixture shaken for 1 min, centrifugation at 4000 rpm for 5 min, supernatant aliquots collection and transfer to Teflon tube containing MgSO4, 30 sec vortexing, centrifugation at 4000 rpm for 2 min; transfer of acetonitrile layer into clean flask and then evaporation near to dryness.

The method provides a relatively clean extract, but still additional SPEs (solid phase extractions) or d-SPES (dispersive solid phase extractions) (Lehotay, 2011) are required to completely purify it (Megson et al., 2016). D-SPE offers QuEChERS the possibility to work with smaller samples and reduced amounts of sorbent and solvents. In this way it is more cost-effective. (Molina-Ruiz et al., 2015; Munaretto et al., 2013). Furthermore, d-SPE removes the evaporation steps and diminishes sample preparation time and effort.

Clean-up techniques

Since the extraction techniques are not selective, a clean-up of the fish fat extract is usually necessary prior compound quantification (Ros et al., 2016). The separation of analytes from lipid interference that could affect the quantification step is frequently based on SPE (Rubio & Perrez-Bendito 2009) or gel permeation chromatography (GPC) (Wu et al., 2011).

For SPE, analytical chemists use column chromatography or commercial already filled SPE cartridges (Santhi et al., 2012) of different sizes. First, n-hexane is used to wet and rinse the column (Akoto et al., 2016; Bhuvaneshwari & Rajendran 2012). The impure extract is transferred to a column of silica or alumina adsorbent (Botaro et al., 2011) and soon after that, an eluent is added in small portions. This eluent can be

n-hexane (Bhuvaneshwari & Rajendran 2012; Kafilzadeh et al., 2012), acetonitrile (Castillo et al., 2012) or mixtures of solvents such as hexane:acetone (Akoto et al., 2016; Santhi et al., 2012), hexane:dichloromethane (Botaro et al., 2011). The solvents move along the column trapping different components of the extract in the stationary packing material or in the mobile phase. Depending on their polarity, molecules partition to different areas and flow through the column in different rates. The eluates are collected and concentrated to dryness.

On the same principle but using Florisil columns, Arzi et al., (2011) and Enbaia et al. (2014) eluted pesticide residues in fractions with petroleum ether and petroleum ether:diethyl ether with slightly different rations. Azab et al., (2013) used solvents like n-hexane, 30% methylene chloride in n-hexane and methylene chloride with good results. Sethuraman et al. (2013) made a treatment firstly through Florisil clean-up and then fractionated the extract using silica gel packed in glass columns. The first fraction was eluted with hexane and the second fraction with 20% dichloromethane in hexane.

Due to so many choices given when working with SPE: columns, cartridges, different adsorbents and eluents the method development takes time and the very complex working steps are difficult to master. The SPE method works well for a variety of matrix, it is very selective and has a concentration effect that contributes to high recoveries. It is also highly reproducible.

GPC uses organic solvents and a hydrophobic gel to separate analytes from other co-extracted lipid molecules based on their molecular size. The separation is made in the column of an automated GPC Clean-up System with large molecules eluting faster from the gel, followed by smaller molecules. Cyclohexane or hexane:ethyl acetate, acetone:cyclohexane or dichloromethane are popular eluents usually used at flow rates of 4-5 ml per min on Bio-Beads S-X3, Envirobead S-X3 Select gels (Streck et al., 2008; Zhang et al., 2012; Wu et al., 2011).

Automation is an advantage, but unfortunately the columns have limited capacity and overloading them could lead to false results. The special equipment itself and the gel column replacement involve costs which greatly limit its popularization. Another issue appears if the fish extract has a big volume of lipids. In this case, the sample has to be split in several parts when size exclusion chromatography (SEC) type GPC is performed. Not injecting the whole quantity of sample and systematical losses of compounds in the GPC system could lead to low recoveries (Sorensen et al., 2015).

GPC has the same high reproducibility but higher solvent consumption if compared to SPE. The separation analyte-lipids is not always complete because of the similarity in molecular sizes (Castillo et al., 2012). One solution to this problem stands in the coupling of two GPC columns in serie (Sorensen et al., 2015). Another solution is switching to other techniques such as the accelerated membrane-assisted clean-up (AMAC) (Streck et al., 2008). AMAC has a better lipid removal capacity than the GPC, reduces solvent waste and saves time.

A daring approach among the purification techniques is the development of an automated high pressure liquid chromatography (HPLC) clean-up for OCs and PCBs (Cioca et al., 2017b). The overall technique is still to be optimized from the perspectives of analyte recovery, reduction of process time and solvent consumption. In order to be more efficient, Kodba & Vončina 2007 combines extraction and purification in an original single-step method that can separate 26 OCs and 6 PCBs from fatty foods of either animal or vegetable origin.

The possible benefits and drawbacks of using a particular extraction or clean-up technique are summarized in Table 1.

Analytical instruments

The first and most popular techniques used in POP determinations are the chromatographic techniques (Andreu & Picó 2012; Bhadekar et al., 2011). In the chromatographic system, the analytes move between two phases and get separated from each other because of the difference in their distribution co-efficient (Bhadekar et al., 2011). The two phases are: the stationary phase and the mobile phase. The stationary phase is located in the system's column and can be a gel, a solid or a mix between the two. Until now, separations have been achieved on cross linked methyl silicon gum columns, fused silica capillary columns, quartz capillary columns and other dedicated columns found on the market (Enbaia et al., 2014; Shi et al.,

Table 1. Advantages and disadvantages of different extraction and clean-up techniques

Technique	Advantages	Disadvantages	Ref
Soxhlet Extraction	- the method is flexible (different solvent systems allow selective extraction of analytes); - high extraction recoveries for large sample sizes; - the extraction of a large quantity of matrix up to 100 g.	- handling; - requires a large quantity of solvent; - large extraction time; - expensive; - physical limitations in achieving miniaturization of Soxhlet equipment.	LeDoux, 2011; Letellier & Budzinski 1999; Sorensen et al. 2015
Ultrasonic Extraction	- decreased extraction time and solvent consumption; - higher penetration of chosen solvent into cellular material and enhanced release of cell content in medium.	- high power consumption; - difficult to scale up.	Ivanovs & Blumberga 2017
Microwave Assisted Extraction	- fast and uses low quantities of solvent (when using a closed vessel); - the possibility to perform the extraction of several samples at the same time; - fast, uses low quantities of solvent and pressure is secure (at atmospheric pressure); - it is possible to couple filtering owing to a cartridge and reconcentration under microwaves.	- time of cooling; - requires filtration; - requires large solvent volumes (100 mL/sample); - expensive; - high power consumption; - heating affects only polar solvents and/or materials; - difficult to scale up; - heat generation, which can lead to unsaturated fatty acid oxidation; - low efficiency when using volatile solvents.	Ivanovs & Blumberga 2017; Letellier & Budzinski 1999; Sorensen et al. 2015
Accelerated Solvent Extraction	- offers better recoveries of all targeted analytes than both Soxhlet Extraction and Ultrasonic Extraction; - short extraction time and low solvent consumption (comparing to classical extraction methods e.g. Soxhlet); - easy to use; - sample preparation for extraction is simple and rapid; - high pressure during extraction allows the extraction of thermally labile analytes, even at high temperatures of this process; - a large variety of solvents can be used with this technique (single solvents or mixtures); - fully automated; - high reproducibility of the extraction parameters; - allows the extraction of at least 24 samples in one cycle.	- the purchase cost of the equipment (apparatus and spare parts) is higher than that of the standard Soxhlet method; - standardized systems typically create large solvent volumes, even when sample sizes are miniaturized; -generally the extraction is not selective (temperature and pressure have a little influence on selectivity); - extract clean-up is usually necessary before final analysis (sensitivity and resolution of chromatographic analysis rapidly deteriorate if the extracts are not purified); - sometimes obtaining different extract volumes due to perturbations of the static valve of the instrument; - complicated cleaning procedure of the ASE cells.	Giergielewicz-Możajska et al. 2011; LeDoux, 2011; Munaretto et al. 2013; Sorensen et al. 2015
Supercritical Fluid Extraction	- offers good quality extracts (very pure) in a short time; - offers minimized product degradation; - eliminates solvent residues (no need for organic solvent); - free of heavy metals and inorganic salts; - no chance of polar substances forming polymers; - high yield; - low operating temperatures (40–80 C°); - a more selective extraction which also provides a faster reaction kinetics than most liquids.	- difficult optimization; - high apparatus and maintenance cost; - high blank and noise levels; - CO_2 is highly selective – no polar substances are extracted; - supply of clean CO_2 needed; - high power consumption.	Ivanovs & Blumberga 2017; LeDoux, 2011; Munaretto et al. 2013
QuEChERS	- excellent performance; - no interferences from co-extractives; - no time consuming evaporation steps or cleanup using traditional SPE in cartridges.	- commercially available QuEChERS kits include containers made of plastic which should be avoided when using organic solvent due to the leaching of plasticizers that may contaminate the sample extracts; - care should thus be taken to replace plastic equipment with appropriate glass or Teflon versions.	Munaretto et al. 2013; Norli et al. 2011; Sorensen et al. 2015
Dispersive Solid Phase Extraction	- effective for lipid rich matrices; - comparing to SPE, dSPE is quicker, requires less manual attention, is more cost effective due to the potential reduction in sample size, sorbent amount, solvents and waste.	- challenges due to co-extraction of the lipids with the target analytes; - the inability to change solvent between the extraction and clean-up steps.	Sorensen et al. 2015

Solid Phase Extraction	- offers clean extracts; - easy to automate; - offers high recoveries for polar compounds; - time and cost efficient; - very reproducible; - polarity-based fractionation of the analytes.	- dissipation of the sample by mixing with the support material over a large surface area; -the sample may not be homogeneously distributed through the SPE column as the process is completed manually surface area.	Sorensen et al. 2015
Gel Permeation Chromatography	- very reproducible; - a good technique for the separation of low molecular mass compounds (up to 400 μm) such as pesticides from high molecular mass compounds such as lipids (600–1500 μm).	- involves instrumentation; - time consuming; - solvent consumptive; - coextracted compounds, including remaining trace amounts of lipids, can reach the GPC eluate and then interfere with the subsequent analysis; - complex samples such as fish, meat, and other fatty matrix extracts often require a two-step clean-up combining GPC and adsorption chromatography in series; - separation of free fatty acids from target analytes is not always possible when there is similarity in molecular size (analyte loss); - using GPC on automated systems makes it difficult to inject more than 90% of the sample, immediately introducing a 10% lower recovery than optimal.	LeDoux, 2011; Norli et al. 2011; Sorensen et al. 2015
Accelerated membrane-assisted clean-up	- automated; - beneficial tool for non-target or effect analysis (separates analytes and matrix compounds mainly by size); - applicable for processing large amounts of sample as a result of the high matrix removal capacity.	- the purchase cost of the equipment (apparatus and spare parts) is high	Streck et al. 2008

2013; Zamir et al., 2013). The mobile phase can be a gas or a liquid, therefore resulting in either a gas chromatography (GC) system or a liquid chromatography (LC) system. The gas carrier is usually Nitrogen (Akoto et al., 2016; Arzi et al., 2011; Azab et al., 2013; Ibigbami et al., 2015; Sethuraman et al., 2013; Veljanoska-Sarafiloska et al., 2011). Helium and Argon are also mentioned in combination with Nitrogen as make-up gas or alone (Ezemonye et al., 2015; Huertas et al., 2016; Shen et al., 2011, Tsygankov & Boyarova 2015). The liquid carriers can be composed of an aqueous base-distilled water with methanol (MEOH) or acetonitrile and different additives such as acetic acid, ammonium acetate, ammonium hydroxide (Lazartigues et al., 2011; Ros et al., 2016).

In order to detect and quantify residues, any GC or LC must be connected to a detector. Electron Capture Detector (ECD) is often used in association with GC, while Mass Spectrometer (MS) can be coupled to both GC and LC (Liang et al., 2012). The operating parameters for GC-ECD and GC-MS instruments in the analysis from fish matrix are various, depending of the group of the types of OCs and PCBs selected for the study (Table 2 and Table 3).

GC-ECD has a relatively high sensitivity for many compounds, especially volatile or semi-volatile. Matrix interference in case of more complex matrices and low amounts of analytes can however give false positive results when performing on such device. The LC-MS/MS technique is able to measure a wide range of potential analytes (nonvolatile, thermally labile, polar or ionic analytes) from small sample volumes, with improved specificity and short run times (Megson et al., 2016).

A group of less popular, but very powerful instruments are becoming the new trend for the detection and quantification of OCs and PCBs from fish. In this category can be included the following: gas chromatography low resolution mass spectrometry (GC-LRMS), gas chromatography high-resolution mass spectrometry (GC-HRMS), comprehensive two-dimensional gas chromatography coupled to time-of-flight high-resolution mass spectrometry (GC×GC-HRTOF-MS), capillary column high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS), liquid chromatography/high resolution mass spectrometry (LC-HRMS) and ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC/LTQ-Orbitrap-MS (MS2/MS3)) (Table 4).

Regardless of which extraction methods, clean-up methods or analytical instruments for

Table 2. Selection of instrumental operating parameters for GC-ECD

Injector settings	Injection temperature and pressure	Oven program	Detection temperature	Carrier gas (N$_2$/He/Ar) flow rate
splitless mode	250 °C	initial temperature set at 90°C (3 min), ramped to 200°C (15 min) at 30°C/min, ramped to 265°C (5 min) at 5°C/min, ramped to 275°C (15 min) at 3°C/min	300 °C	30 ml/min
-	230 °C	initial temperature at 170°C (8 min), ramped to 200°C (14 min) at 5°C/min	320 °C	1 ml/min
-	260°C	initial temperature at 160°C (2 min) ramped to 240°C (20 min) at 5ºC/min	320°C	3 ml/min
splitless mode	280 °C	initial temperature at 90°C (2 min) ramped at 15°C/min to 130°C, ramped at 4 °C/min to 290°C	320 °C	60 ml/min
-	250 °C	initial temperature at 80°C (1 min), ramped to 180°C at 10°C/min (3 min), ramped to 300°C (2 min) at 10°C/min	300 °C	4.0 ml/min
-	275°C	initial temperature at 191°C (12 min), ramped to 216°C (20 min) at 50°C/min	300 °C	30 ml/min
splitless mode	250 °C	initial temperature at 120°C (2 min), ramped to 180°C at 30°C/min, ramped to 240°C (2 min) at 4°C/min, ramped to 260°C (10 min) at 5°C/min	300 °C	-
-	220 °C	Initial temperature at 150°C (4 min), ramped to 290°C (10 min) at of 8°C/min	300 °C	30–32 ml/min

Table 3. Selection of instrumental operating parameters for GC-MS

Injector settings	Injection temperature and pressure	Oven program	Detection temperature	Carrier gas (N$_2$/He/Ar) flow rate	Mass spectro-meter mode
-	250 °C	initial temperature at 150°C (2 min), ramped to 170°C at 30°C/min, ramped to 200°C(5.5 min) at 4°C/min, ramped to 320°C (10 min) at 30°C/min	320 °C	1 ml/min	SIM
-	250 °C	100 C for 1 min, from 100 to 190°C at 20°C/min (held for 2 min), then to 250°C at 3C/min and finally to 300°C at 50°C/min (held for 10 min)	300 °C	1 ml/min	SIM
-	250 °C	Initial temperature 70°C (held for 1 min), increased to 160°C at a rate of 15°C min1 and thereafter increased to 190°C at a rate of 2°C min1. Finally the temperature was increased to 320°C at a rate of 15°C min1 and held for 5 min.	320 °C	2.25 ml/min	EI and SIM
splitless mode	250 °C	60 C hold for 0 min, ramp at 30 C min1 to 100 C, hold for 3 min, ramp at 3 C min1 to 220 C, hold for 3 min, ramp at 10 C min1 to 280 C, and hold for 10 min	-	1.2 ml/min	EI and SIM

Table 4. Current powerful tools for POPs detection and quantification

	Analytes	Positive aspects	Negative aspects	Ref
GC-LRMS	Mostly PCBs (dl-PCBs)	Sufficient sensitivity Higher limits of detection than with HRMS	Limited number of compounds for simultaneous determination Less selective than HRMS (identification can be less clear)	Webster et al. 2013
GC-HRMS	PCBs	Sensitivity; Non-target approach Post target analysis; Unlimited determination of compounds (in theory) Reduced analysis cost; Improved cost effectiveness; High separation power; Narrow peaks; Short analysis time	More expensive than gas chromatography coupled with ion trap mass spectrometry (GC-ITMS)	Romero-González et al. 2017, Webster et al. 2013. Wang et al. 2015, Zacs et al. 2015
GC×GC-HRTOF-MS.	PCBs	Results in good agreement with the results obtained through GC-HRMS Better chromatographic separation than the one obtained with one dimensional GC Identification of non16 target compounds Simultaneous analysis of various classes of compounds	-	Xia et al. 2016
HRGC-HRMS	OCs and other pesticides	Provides more structural information of the compounds Improved peak shape and screening detection limit Low values of mass accuracy error	-	Munaretto et al. 2016
LC-HRMS	OCs PCBs	Flexible; Effective; Highly selective; Rapid and robust analysis in both qualitative and quantitative applications Sensitivity comparable to that of LC–QqQ-MS	-	Aceña et al. 2015, Strobel et al. 2018
UHPLC/LTQ-Orbitrap-MS (MS2/MS3)	OCs and other pesticides	high sensitivity; low costs; increased sample throughput Improved dynamic range and linearity Results in good agreement with the results obtained through UHPLC-QqQ-MS/MS	-	Farre et al. 2014

quantification are used, the reported analysis protocols for POP determinations from fish matrix exhibit the common features summarized in Fig. 1.

Persistent organic pollutants

It is ideal to detect and quantify as many types of organic residues as possible with one complex method (Cifuentes 2012). Nevertheless in the routine analysis from fish matrix, some OCs and PCBs were more intensely studied then others (Table 5).

Contaminated fish species and sites
Africa

In northern Ghana, Akoto et al., (2016) studied Catfish (*Clarias Anguillaris*), Silver catfish (*Schilbe intermedius*), Trunkfish (*Marcusenius senegalensis*) and Mango Tilapia (*Sarotherodon*
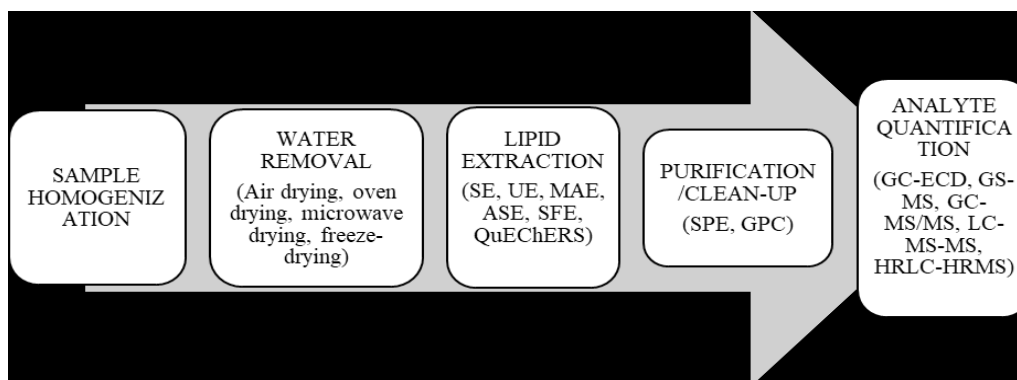
Figure 1. Flowchart for extraction and analysis of fish samples

Table 5. OCs and PCBs analyzed from fish matrix in the last five years

	OCs	PCBs
Frequently studied	hexachlorocyclohexane isomers (β,γ,δ HCH), dichlorodiphenyl trichloro ethane (pp', op'DDT) and its metabolites (pp'DDE, pp'DDD), aldrin, dieldrin, heptachlor, heptachlor epoxide, endosulfan isomers (α, β), endrin, lindane	ΣPCBs
Occasionally studied	metoxychlor, benzene hexachloride (β,γ,δ BHC), hexachlorobenzene (HCB), mirex, endosulfan sulfate, chlordane isomers (-cis, -trans), dicofol (op',pp'), non-achlor (-cis, -trans), bromocyclene, oxychlordane	PCB 31, 52, 101, 118, 153, 180, 194

galilaeus) in Tono Reservoir located at Navrongo and his health risk estimation revealed that aldrin in *M. senegalensis* had great potential for systemic toxicity to consumers. In Lake Hawassa, one of the Ethiopian Rift Valley Lakes, Deribe et al., (2014) found PCBs, DDT and endosulfan concentrations that exceeded the reference dose for children between the ages of 0–1 year in *Barbus intermedius*. Other species of fish -*Clarias gariepinus* and *Tilapia zilli*- collected from three rivers in Edo State, Nigeria revealed high levels of heptachlor epoxide, dieldrin, endrin, aldrin that could have endangered consumers (Ezemonye et al., 2015). In the same southwestern region of the country, *Clarias gariepinus* was analyzed from Ogbese River in Ekiti State. In this location , this species of fish had endosulfan I above the EU and FAO/WHO maximum residue limits (MRL), while some sardines, mackerel and tuna exceeded the MRLs for endosulfan, Heptachlor epoxide and Dieldrin in the area of Tripoli(Enbaia et al., 2014, Ibigbami et al., 2015). In Kenya, Oluoch-Otiego et al., (2016) presents an interesting approach of

the subject and demonstrates the occurrence of moderate to high levels of PCB in fish and their cestode parasites from Lake Victoria. Cestodes such as *Proteocephalaus sp.*, *Monobothrioides sp.*, *Proteocephalaus sp.* and *Ligula intestinalis* bioaccumulated higher levels of PCBs than their hosts - *Oreochromis niloticus*, *Lates niloticus*, and *Rastrineobola argentea* and therefore provide a promising biomonitoring material for PCBs. Azab et al., (2013) conducted analysis on *Tilapia nilotica* fish samples taken from four sites at Manzala Lake, Egypt: Ashtoum El-Gamel outlet, Round road, Bughas El-Rasoah and Port-Said Damietta road. OCs in fish and its ecosystem were significantly higher in the Round road area followed by the Port-Said Damietta and finally, Ashtoum El Gamel. The levels of organochlorines were not higher than the maximum permissible level recorded by FAO/WHO and the public was at that time not at serious risks with fish consumption.

Europe

Studies from arbitrary European countries show that POPs are especially present in fish

collected in either lakes or sea sites. In Romania, the pesticide determination from anchovy collected from the coastal romanian Black Sea revealed high values for p,p′-DDE, lindane, heptachlor and PCB 28 (Galațchi et al., 2017), but remained below the maximum values allowed by national legislation (Order No. 147/2004). In Spain, analysis conducted on european catfish (*Silurus glanis*) living in Ebro river areas that are under the influence of a chlor-alkali plant, such as Sau, indicated highlevels of DDTs (mainly 4,4′-DDD and 2,4′-DDD) (Huertas et al., 2016). Even though the detected OCs or PCBs from European countries are many times not evidenced in dangerous concentrations, still as persistent organic pollutants with bioaccumulation particularities, they represent quite high risk for fish, fish foods and consumers (Veljanoska-Sarafiloska et al., 2011).

Asia

Some fish and seafood from different sites in Asia present low residue levels- below MRLs, but still their long-term consumption can cause chronic toxic effects or lifetime cancer risks. Santhi et al., (2012) declares a negligible health risk caused by dietary intakes of OCs along with mackerel (Scomberomorus commerson), sea bass (*Lates calcalifer*), stingray (*Dasyatis sp.*), red snapper (*Lutjanus malabaricus*). While most of the chemical compounds reveal low concentrations attributed to a past use of pesticides in samples from Peninsular Malaysia, an antagonistic situation is revealed by Zamir et al., (2013) in Bangladesh, a country which is actually known to import fish and seafood to Malaysia. The ΣDDTs/DDT value (0.59, 0.68 and 0.57 %) in fresh rui, katla and pangus from the country's multiple bazars and markets, indicates the recent and ongoing use of dangerous POPs in Bangladesh. Other species such as mackerel, tuna, hairtail, yellow croaker and eel analyzed from Busan (largest fish market in South Korea) were found to mostly contribute to the dietary intake of especially total DDTs, HCBs, total chlordanes and PCBs (Choi et al., 2016; Moon et al., 2009). Some serious concerns have been expressed by Shi et al., (2013) and Qian et al., (2017) regarding the consumption of fish from Shantou Harbor, Haimen Bay and Xiamen, China. In these areas, the levels of PCBs, DDTs and HCHs are remarkable and can cause great health risks for the local population that serves this kind of food on a daily basis. In Iran, Arzi et al., (2011) studied

Benni fish from three areas (Shadegan, Mahshahr and Susangerd) and discovered that regardless to the kind of pesticide, Mahshahr and Susangerd Benni fish were the most, respectively the least contaminated fish. The highest values recorded were for o p′-DDT and HCH with no mentioning of maximum limit surpass. One year later, Kafilzadeh et al., (2012) tests the *Barbus brachycephalus caspius* fish for OC residues in a southern region of the same country at Lake Parishan and his results point out that the pollution of the lake represents a danger to both aquatic organisms and humans. In India, Mahboob et al., (2015), collected *Catla catla* samples from the River Ravi where the pollution levels in the river discharge are reportedly very high due to careless disposal of large amounts of industrial and agricultural wastewater and faulty drainage system in both India and Pakistan. The concentration of endosulfan, carbofuran, and deltamethrin were higher than the permissible limits for fish set by international agencies and therefore, the *Catla Catla* fish species poses a potential ecological risk to the aquatic ecosystem and a consequent hazard to human health. In fish collected from Mumbai, India, at Dadar market, the residual concentration of pesticides such as DDTs or HCHs was well below the tolerance limits (Sethuraman et al., 2013). An OC determination was accomplished by Bhuvaneshwari & Rajendran 2012 on *Etroplus Suratensis*, *Oreochromis mossambicus*, *Liza parsia*, *Channa striatus* and *Silurus wynaadensis* samples that were collected at various locations in River Cauvery and in Veeranam Lake from India, Asia. The assessment on the carcinogenic risk associated with fish consumption revealed that these species may pose carcinogenic risk to the local population.

North America

In a study from 2008, de Vlaming integrates the results of the POPs determinations from fish collected from some Central Valley and Sacramento/San Joaquin River Delta Estuary water bodies between 1970-2003. The level of pollution with OCs and PCBs was revealed to be at concentrations of concern for human health.

South America

Oreochromis niloticus were collected from four fish farms in three different states in Brazil. Two types of culture systems were investigated: net cages and intensive tank systems. The first type were located near a large hydroelectric dam

in Southern Brazil and near a hydroelectric dam in the Tiete River basin, where the main economic activities conducted nearby are dairy and livestock production and sugar cane production. The intensive tanks were located one in São Paulo state, close to an alcohol plant, and is surrounded by sugar cane plantations and the other in a rural zone in the Muriae River basin, where mining and sand extraction are the main anthropogenic activities. All these fish farms are responsible for the production of significantly high amounts of fish, in the tilapia production context in Brazil. No samples exceeded international maximum limits for safe fish consumption. Sum of DDTs was the predominant pesticide in fish muscle, found in all fish samples. Slightly higher OC concentrations were observed in adults. Among the rearing systems, net cage fish presented higher lipid levels and, consequently, higher OC concentrations than fish from intensive tank farms. Some OCs (Sum of HCHs, Aldrin, Dieldrin and Endrin) presented strong positive correlations (p < 0.05) between feed and fish muscle concentrations (Botaro et al., 2011).

Conclusions

Currently, methods like ASE, SFE and QuEChERS quickly take over the old, classical SE due to their great advantages such as automation, low solvent consumption and efficiency of the extraction process. More expensive equipment, type ASE or SFE, can be a favourable choice for official control laboratories which need to analyse a great number of samples in a fast and easy manner. SPE with silica, alumina or Florisil packed columns persist as the most popular clean-up procedures in the determination of POPs from fish matrix, followed by the costly GPC and other extraction-purification one step methods. GC-MS/MS and LC-MS/MS are the tools of the future for POP residue quantification.

With numerous possibilities regarding the techniques to choose for fish sample preparation, it is impossible to acknowledge only one or a combination of multiple techniques as a generic approach. A time and cost efficient method is always preferred, but that strongly depends on the equipment already available in laboratories and the context of the analysis.

Part of the constantly measured and monitored POPs are DDT and its metabolites, HCH isomers,

HCB, lindane, aldrin, dieldrin and others which were very often used in the past and are banned at present due to the harm they might cause to humans and environment.

A higher percentage of these compounds, most of the times exceeding MRLs, are found in fish from third world country waters from Africa and Asia as a result of illegal chemical waste dumping and low control of the situation. The levels are lower on the other continents, but residues still exist in fish from lakes, seas and oceans which once again prove the huge issue of bioaccumulation and the power of POPs years after banning.

Compliance with ethical standards
Author Ana-Andreea Cioca declares that she has no conflict of interest.
Author Olaf Heemken declares that he has no conflict of interest.
Author Liora Mihaela Colobatiu declares that she has no conflict of interest.
Author Marian Mihaiu declares that he has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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