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Review

Analysis of synthetic endocrine-disrupting chemicals in food: A review

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ABSTRACT

This work focuses on a revision of analytical methodologies for the determination of industrial chemicals that have an endocrine-disrupting effect on food commodities. These food commodities have been divided into two major categories: crops and food of animal origin. The reviewed methods have been commented on in terms of sample preparation, analytical methods, and the occurrence of the studied compounds.

Contents

1. Introduction

An endocrine disruptor chemical (EDC) is an exogenous substance, or a mixture, that alters the functioning of the endocrine system and, consequently, causes adverse health effects on an intact organism [http://ec.europa.eu/environment/endocrine/documents/studies_en.htm], or its progeny or (sub) populations. It interferes with the body's ability to regulate growth, its development, metabolism or other functions. There are hundreds of EDCs in the environment, in food and in consumer products. They include persistent organic pollutants, pesticides, some heavy metals, preservatives and fragrances, industrial chemicals and their by-products or waste, as well as plant-derived compounds. EDCs can contribute to a wide range of diseases and disabilities, including obesity, diabetes, cancer, heart disease, reproductive health problems along with neurodevelopmental and neurodegenerative

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disorders [1]. Effects on reproduction and on the immune system have been reported in fish, alligators, seals and birds [2], but evidence of their effects on humans is varied and sometimes contradictory.

One area of endocrine disruption, which is the subject of huge debate, is the issue of low dose effects. What constitutes a 'low dose' is not always clear, but it's generally considered to be a dose which is far lower than that normally expected to have an effect [72].

As low dose research is often looking for subtle, but potentially significant changes, there have been major problems with reproducibility. An example of the low dose effect is the research that was done by Richard Sharpe's team [73] at Edinburgh University, which initially showed that exposure to octylphenol (OP) or butylbenzyl phthalate (BBP) on rats in the womb led to testicular weight reduction. There are two particularly important aspects to low dose effects:

The inherent sensitivity of the endocrine system and development.

The interaction between the endocrine and developmental systems is extremely complex and sensitive. Science does not yet properly understand the operation and interaction of these systems, so it is not possible to properly evaluate the impact of chemicals on them. It is quite possible that very low doses of chemicals could have very significant effects.

2. Additivity and synergism

The addition of a small quantity of a chemical to the body may have effects because it acts in addition to another chemical which is already there. For example, an estrogen such as nonylphenol could act in addition to a natural estrogen that is already present.

In **synergism,** the combination of two chemicals results in more than an additive response - for example one chemical might lead to an increase in the number of receptors in a cell, whilst the second chemical binds and activates them.

EDCs have been highlighted as a result of the publication of *Our Stolen Future* [3] and *Assault on the Male* [4], which warned of the health effects of many man-made chemicals, which are suspected of interfering with the endocrine systems of both humans and wildlife. This was the case with dichlorodiphenyl trichloroethane (DDT), following *Silent Spring* in 1962 [5], which warned of DDT's threat to ecosystems. Since the impact of *Our Stolen Future*, the general public has become worried about the health risks connected to EDCs and proper regulations for EDCs have become government requirements.

Certain EDCs are sometimes referred to as estrogen mimics. The incidence of cancer is increasing steadily in tissues that are regulated by estrogens. It has been proposed that not only increased cancer incidence but also fertility problems in humans and wildlife are caused by an increased exposure to compounds that mimic endogenous estrogens [6].

In recent years, various international and national governmental bodies, and non-governmental organizations, have published lists of suspected endocrine-disruptors. The Institute for the Environment and Health has developed a database of these lists, together with further data extracted from original published studies or review articles. This database contains a total of 966 compounds, or elements purported to be EDCs.

One of the sources used in the compilation of the IEH database, is the BKH report from the European Commission, entitled *Towards the Establishment of a Priority List of Substances for Further Evaluation of Their Role in Endocrine Disruption*. It is a list of 564 chemicals either known or suspected of being endocrine disruptors, prioritized in terms of their potential for human and wildlife exposure, their high production volumes (HPV) and/or persistence as well as the strength of scientific evidence on their having endocrine-disrupting properties. The listings produced are not regarded as final and unchangeable: addition or removal of chemicals may be required in response to either developments in scientific knowledge or changes in chemical usage patterns [http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm#priority_list].

They are classified into three groups:

Group I. Compounds for which there is considered to be evidence of endocrine-disrupting activity and for which a high level of concern exists with regard to exposure.
Group II. Potential endocrine disruptors or compounds for which there is a medium level of concern with regard to exposure.
Group III. Compounds for which there is considered to be insufficient evidence of endocrine disruption, or for which there is only a low level of concern with regard to exposure.

The literature on the analysis of the different groups of toxic compounds in food is extensive, and this review will not do justice to all the available information; we have focused on selected groups of industrial EDC chemicals, those which are the most important and widely studied. The selected groups of compounds are Polychlorinated biphenyls (PCBs), Polybrominated diphenyl ethers (PBDEs), Dioxins and Furans, Phthalates, Parabens, Bisphenol A (BPA), Octylphenol and Nonylphenol, and additionally, Perfluorinated compounds.

Food samples are very complex matrices and the determination of contaminants at low concentrations within them is a difficult task. Depending on the kind of matrix, the sample handling can be different, involving different pre-treatment and extraction strategies before analysis. In general, differences are marked depending on whether samples are liquid or solid, or if the fat percentage or water percentage is high or low. The extraction of endocrine-disruptor compounds in food is followed by analysis and analyte quantification. In general nowadays, the determination is performed using chromatographic techniques coupled to a mass spectrometer system. Depending on the features of the compounds, the analytical techniques chosen will be based either on gas or liquid chromatography. The most common analytical methodologies for the determination of EDCs in food commodities are schematized, taking into account if the matrix is solid or liquid; and structured into four different steps: pre-treatment, extraction, clean-up and instrumental analysis.

The main way in which most of industrial, chemical endocrine disruptor compounds such as Bisphenol A and phthalates come in contact with food as contaminants is through *food contact materials*, including packaging materials, but also cutlery, dishes, processing machines, containers etc. European legislation limits the concentration of potential mutagens, or EDCs, in food which is in contact with plastic. The legislative documents concerning food contact materials are available directly from the Commission Services, DG SANCO [71].

Grob et al. [74] reviewed the regulations and use of endocrine-disrupting compounds (EDCs) in food packaging and discussed their presence within the context of new toxicology paradigms—the core hypothesis of this review is that chemicals leaching from packaging into food contribute to human EDC exposure and might lead to chronic diseases, in light of current knowledge.

The transfer of constituents from food contact materials to food is called migration. To ensure consumer health protection and to avoid any contamination of food staff, two types of migration limits have been established for plastic materials: an Overall Migration Limit (OML) of 60 mg (of substance)/kg (of foodstuff or food simulants)—this applies to all substances that can migrate from food contact materials to foodstuffs; and a Specific Migration Limit (SML), which applies to individual authorized substances and

Table 1
Information of the selected EDC groups and individual chemicals.

Groups of EDCs	Use	Target matrices/human exposure	Chemical contaminant	Notes
INDUSTRIAL CHEMICALS				
Phthalates	Production of plastics and resins	Vegetable oils, chocolate, cookies, snacks, milk, cheese, butter, wine, beer, pizza, baby-food/Migration from food packaging. Carry-over to ruminants from contaminated pastures	Dimethyl phthalate (DMP)	Group III
			Diethyl phthalate (DEP)	Group III
			Di-*n*-butyl phthalate (DBP)	**Group I** Low to medium persistent, bioaccumulated at low trophic levels. High Production Volume[a]
			Butyl benzyl phthalate (BBP)	**Group I** High Production Volume[a]
			Butyl decyl phthalate (BDP)	
			Di(2-ethylhexyl) phthalate (DEHP)	**Group I** Persistent, bioaccumulated High Production Volume[a]
			Di-*n*-octyl phthalate (DOP)	Group III
			Diisononyl phthalate (DINP)	Group II
			Isobutyl phthalate (iBP)	Group III
Alkylphenolic Compounds	Variety of uses, including surfactants, antioxidants and PVC additive	Cereal, crustacean, mollusc, fish, beverages, baby-food, meat/Release from polystyrene and PVC in packaging material and bottles. Carry-over to fish and animal from contaminated pastures or water. In food packaging, nonylpnhenol originates from oxidation of the antioxidant additive trisnonylphenyl phosphite (TNPP)	Octylphenol polyethoxylates (OPEs)	Group III
			Octylphenols (Ops)	Group III
			Nonylphenols (NPs)	Group II High Production Volume[a] Expected to bioaccumulate
			4-tert-butylphenol (t-BP)	Group III
			4-nonylphenol (NP)	Group III
			4-tert-octylphenol (4-t-OP)	Group II High Production Volume[a] Expected to bioaccumulate
Phenols	Production of plastics and resins; preservatives, disinfectants	Cereal, fish, meat, milk, canned food and beverages, honey, baby-food/Migration from packaging as coating in food cans	Bisphenol A (BPA)	**Group I** Persistent High Production Volume[a]
			BPA diglycidyl ether (BADGE)	Group II
			Bisphenol F (BPF)	Group III
			Bisphenol B (BPB)	Group III
			Ortho-phenylphenol (OPP)	Group II
			2,5-dichlorophenol	Group III
Polychlorinated biphenyls (PCBs)	Past widespread industrial use (electrical equipment, hydraulic systems, in plastics, coats, glues, paints, etc.); PCB are severely restricted and banned; still available through existing products	Crustaceans, fish, mussel, vegetables, vegetable oils, cereals, meat, hens and pork fat, milk, cheese, butter, margarine, eggs/Exposure indirectly through food (fish) and mother milk by emission through the waste stage. This substance is stored in animal fats	PCBs	**Group I** (PCB 47, 77, 153 and 169) Group II (PCB 48, 61, 75, 136 and 156) Group III the rest. Persistent, bioaccumulating compounds
Polybrominated diphenyl ethers (PBDEs)	Flame retardants used in textiles, plastics, building materials, and electronic equipment. PBDEs have been banned in several countries	Crustaceans/Exposure indirectly through food (fish and meet) by emission through the waste stage. This substance is stored in animal fats	PBDEs	Group II and III Persistent, bioaccumulating compounds
Dioxins and furans	Forming during combustion (municipal waste incineration), metal, paper and pulp production, chlorophenols and herbicides	Crustaceans, fish, meat, butter, milk, vegetable oils, fat and liver of hens, eggs/ Exposure through emission at production and at waste stage (incineration); From there they pass up the food chain into meat, fish and dairy products	Polychlorinated dibenzo-p-dioxins	**Group I** (1,2,3,7,8-PCDD and 2,3,7,8-TCDD) Group III the rest Persistent, highly accumulating
			Furans (Fs)	**Group I** (2,3,4,7,8-PCDF) Group II and group III the rest Persistent, highly accumulating
Polycyclic aromatic hydrocarbons (PAHs)	Variety of anthropogenic sources, including fossil fuel combustion, municipal and industrial effluents, creosote, oil spills, urban	Smoked meat and fish, crustacean, meat, butter/ Exposure through smoked meat and fish and indirectly food might be contaminated	PAHs	Group III Persistent, bioaccumulating compounds

Table 1 (*continued*)

Groups of EDCs	Use	Target matrices/human exposure	Chemical contaminant	Notes
	and agricultural runoff, asphalt production, and waste incineration	by PAHs through the environment or via formation during processing		
PESTICIDES				
Organochlorines	Insecticide mostly on non-food crops (used to control termites, and on home lawns and gardens); forbidden in EU and US. *Stable metabolite-oxychlordane*	Vegetable oils, fish oil, pork fat, eggs, meat, milk, cheese, butter, tea, cocoa, coffee, vegetables, fruits/Found in mother milk; due to atmospheric transport, Inuit women tend to have a diet highly contaminated with chlordane	Chlordane	**Group I** Persistent, bioaccumulation observed.
	Insecticide banned in all countries for use in agriculture. Still used in disease vector control (eg. Malaria)—recommended for indoor spraying only	Fruits, vegetables, rice, eggs, milk, butter, fish fat/ Widespread persistence in environment, biota and mother milk and food. Exposure has occurred due to eating contaminated fish and animals	DDT	**Group I** Persistent, bioaccumulated High Production Volume[a]
	Insecticide on seed and soil before food crops are planted. Also used as a timber treatment, and in the home, for head lice	Olive oil, vegetables, fruits, milk, butter, cheese, meet, chocolate, cereals, baby-food/Long range transport seen. Observed in biota and water systems, through wastewater at production and through application on soil and seeds	Lindane (Gamma-HCH)	**Group I** Inherently biodegradable, bioaccumulated High Production Volume[a]
Organochlorines	Fungicide on seeds and food crops; Severely restricted in the EU but still used in some parts of the world	Vegetable oils, fish oil and fat, pork fat, vegetables, fruits, rice, eggs, baby food, milk, butter/Long range transport. Found in environment and biota. Exposure through production at industrial wastewater	Hexachlorobenzene (HCB)	**Group I** Persistent, biocaccumulated High Production Volume[a]
	Insecticide and acaricide Widely used in developing countries	Olive oil, olives, vegetables, fruits, fruit juices, eggs, baby-food, butter, milk/ Exposure through contaminated animals, fruits and vegetables.	Endosulfan (alpha, beta and sulfate)	Group II Persistent, biocaccumulated
	Acaricide Can be contaminated with alpha-Cl-DDT. In EU, dicofol is not permited if it contains less than 78% of pp dicofol or more than 1 g/kg DDT and related compounds.	Vegetables, fruits/Exposure through contaminated fruits and vegetables. Found in human body fat	Dicofol	Group II
	Insecticides Endrin also avicide and rodenticide. Dieldrin-banned	Vegetable oils, fish oil and fat, pork fat, fruits, vegetables, eggs, milk, butter, baby-food, meet/ Exposure through contaminated vegetables, fruits and animals (at production-wastewater, and application)	Heptachlor	Group II
			Dieldrin	Group II Persistent, bioaccumulated
	Aldrin can break down to dieldrin	Aldrin	Group II	
			Endrin	Group II
Organophosphates	Insecticides Pesticides- diazinon and chlropyrifos	Olive oil, fruits, vegetables, rice, wheat flour, fruit juices, wine, baby-food, meat, milk/	Acephate	Group III
			Dimethoate	Group II
			Malathion	Group II
			Parathion	Group II
			Methylparathion	Group II
			Diazinon	Group II
			Chlropyrifos	NA

Table 1 (*continued*)

Groups of EDCs	Use	Target matrices/human exposure	Chemical contaminant	Notes
Herbicides	Amide herbicide also on food crops	Vegetables, fruits, rice, milk, meat/Exposure through contaminated vegetables, fruits and animals	Acetochlor	**Group I** Slightly bioaccumulated High production volume[a]
			Alachlor	**Group I** Slightly bioaccumulated High Production Volume[a]
	Triazine herbicide on food crops and alongside roads and uncultured land	Olive oil, butter, fruits, fruit juices, vegetables, eggs, raisins, milk, baby-food, meat/Contaminated soil alongside roads. Exposure through contaminated vegetables, fruits and animals	Atrazine	**Group I** Persistent High Production Volume[a]
			Simazine	Group II
	Urea herbicides on food crops	Olive oil, fruits, vegetables, raisins, cereals, meat/	Linuron	**Group I**
	Linuron- biodegradable into metabolite 3,4-*DCA*	Exposure through contaminated vegetables, fruits and animals (at production-wastewater, and application)	Diuron	High Production Volume[a] Group II
	Diphenyl ether herbicide	Vegetables, eggs/Exposure through contaminated vegetables and animals	Nitrofen	Group II
	Phenoxy herbicide	Human milk, barley/ Exposure through contaminated vegetables and animals	2,4-Dichlorophenol (2,4-D) *Pesticide intermediate*	Group II
Fungicides	Fungicide on fruit and food crops	Fruits, vegetables wheat flour, wine, raisins, olive oil, baby-food/Exposure through contaminated vegetables, fruits and animals (at production-wastewater, and application)	Maneb	**Group I** High Production Volume[a]
			Zineb	**Group I** High Production Volume[a]
	Fungicide on fruit, vegetables and ornamental plants	Fruits, vegetables/Exposure through contaminated vegetables, fruits and animals (at production-waste stage, and application)	Vinclozolin	**Group I** High Production Volume[a]
	Fungicide on fruits and food crops	Fruits, vegetables, wheat flour, wine, rice/Exposure through contaminated vegetables and fruits	Carbendazim	Group II
			Iprodione	Group II
			Prochloraz	Group II
			Triadimefon	Group II
Pyrethroids	Insecticides widely used in agriculture	Olive oil, olives, butter/ Exposure through contaminated food	Cypermethrin	Group III
			Deltamethrin	Group III

Reference Sources: Institute for Environment and Health (IEH); BKH report for the European Commission (EC-BKH).
Abbreviations: PCDF-pentachlorodibenzofuran; PCDD-pentachlorodibenzodioxin; TCDD-tetrachlorodibenzo-p-dioxin.

[a] The selection of High Production Volume chemicals was based on the HPV list from Regulation (EEC) no. 793/93 on chemicals with a production volume of more than 1000 tonnes per year.

is fixed on the basis of the toxicological evaluation of the substance. The SML is generally established according to the Acceptable Daily Intake (ADI), or the Tolerable Daily Intake (TDI), set by the Scientific Committee on Food (SCF). To set the limit, it is assumed that, every day throughout their lifetime, a person weighing 70 kg eats 1 kg of food packed in plastics containing the relevant substance at the maximum-permitted quantity.

As an example, for BPA, the SML is 0.6 mg/kg. In the case of phthalates, which are also restricted in the EU, in particular for phthalic acid, bis(2-ethylhexyl) ester (DEHP), the SML is 1.5 mg/Kg.

Other industrial endocrine-disruptor chemical compounds can come into contact with food in different ways apart from packaging but their presence in food is also regulated—as an example, the maximum concentration of PCBs and Polyclhorinated dibenzo-p-dioxins and furans (PCDD/Fs) are regulated by Commission Regulation (EC) No 1881/2006 [45]. The maximum concentration of Polycyclic Aromatic Hydrocarbons (PAHs) in food is also regulated by this directive Table 1.

3. Analysis of industrial EDC chemical in food commodities

In general, food commodities can be classified into three main groups: crops, which include vegetables, fruits and cereals; products of animal origin and processed food. The characteristics of food included in these groups are widely varied and general

trends are complicated to establish. Furthermore, there are a variety of studied EDCs which complicate this objective.

In the present work, a group of publications concerning the analyses of industrial endocrine-disruptor chemical compounds in crops and in animal-origin products has been selected and analytical methodologies and occurrence has been commented on.

In the European Guideline for Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed [7], ten types of matrices are considered: high water content (most fruits and vegetables, such as tomato, lettuce, leek, mushrooms etc.), high oil content (vegetable oil, olives, avocado), high protein content and/or starch content and low water and fat content (cereals, rice), high acid content and high water content (lemons, grapes, pineapple etc.), high sugar and low water content (honey, dried fruits),"difficult or unique commodities" which include coffee, tea, spices; meat and seafood; milk and milk products; eggs; and fat from food of animal origin. In all cases, a clean-up stage is often necessary to remove undesirable matrix components such as pigments (chlorophyll, carotenoids), triacylglicerides, lecithin etc. Probably, the most complex matrices are those with a high-fat content; this is because it is quite complicated to extract the EDCs without co-extraction of lipids, which are usually difficult to remove from the extract and may harm the detection system.

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), as well as polychlorinated biphenyls (PCBs) constitute three classes of structurally-related chlorinated aromatic compounds that represent potential risks for human health. PCDDs/Fs are formed as by-products of a wide variety of chemical industry and combustion processes that contain chloride and chlorinated aromatic hydrocarbons. PCDD/Fs are formed as by-products of a wide variety of chemical industry and combustion processes that contain chlorine and chlorinated aromatic hydrocarbons sources; in addition, PCDD/Fs are also associated with chlorine-bleached carton packaging used in direct food contact [75], and PCBs have been widely used as commercial products in industry, [8,9] specifically in pigments on food packaging [76]. Due to their low water solubility, hydrophobic character and resistance to metabolic degradation, these substances are found in a broad range of biological samples, and tend to bio-accumulate in animal and human adipose tissues throughout the food chain [10].

Nowadays, diet is considered as a major source of non-occupational human exposure to PCDD/Fs and PCBs, with foodstuff of animal origin accounting for more than 90% of human body burden [11–14]. Since fish material, or products of vegetable origin such as oils, are by far the largest component in the diet of many animal species, the presence of relatively small concentrations of dioxins and PCBs in these materials is a matter of concern because they can bioaccumulate in fish and meat.

After several dioxin crisis in Europe, the European Union implemented comprehensive regulations on the maximum PCDD/F content of a variety of foods and feedstuffs in order to reduce human exposure to these compounds. Recently, the maximum dioxin levels established by Council Regulation 2375/2001/EC and Council Directive 201/102/EC have been revised to include the contribution of dioxin-like PCBs in dioxin equivalent (TEQ) concentrations [15].

Bisphenol A is an industrial chemical commonly used as a monomer in the preparation of epoxy resins, polycarbonate plastics and as an antioxidant or stabilizer in polyvinylchloride. After BPA has been released into the environment and manufactured into packaging materials, food and feed may contain some of these products as a results of (i) diffuse environmental pollution and direct uptake by animals via food or air; and potential bioaccumulation and transfer through the food web; (ii) food processing coming into contact with plastics, resins, lacquers, surfactants and paints from pipes, gaskets, and containers; and (iii) migration from packaging and bottling material, envelopes, and printer ink. Humans may also be affected through the consumption of contaminated drinking water and foods [16]. Alkylphenols, including OP and NP, are widely used as intermediates to produce surfactant (anionic and non-ion surfactants) and as stabilizers of ethyl cellulose resins, oil-soluble phenol resin and esters. These compounds are also discharged into the environment as metabolites of alkylphenol ethoxylates, mainly by biodegradation from sewage treatment plants. Human exposure to APs and NPs, in particular, can occur by different routes, such as inhalation, ingestion of contaminated food and dermal absorption. The oral intake may be significant, e.g. via seafood, water supply or food contamination.

Nonylphenol and Bisphenol A are lipophilic compounds. Therefore, they can easily contaminate foods of animal origin, which are thought to represent the most important source of human exposure to many organic pollutants. Knowledge of animal tissue concentrations is important to understand the potential risk to animal health and performance, as well as the risk to human health.

Phthalates have been chosen because they give great cause for concern regarding bioaccumulation (accumulating in living tissues and in the food chain). They are poorly biodegradable and are potentially toxic. Due to man's activities, they are present in the environment in quite large quantities; they have been used as plastifying agents, mainly to make polyvinyl chloride (PVC) supple and flexible.

Phthalates have been used as plasticizers in many plastics since the 1930s, with a quarter of the total plasticizer ever produced being diethylhexylphthalate. Phthalates used include diethylhexylphthalate (DEHP), monoethylhexylphthalate (MEHP), dimethylphthalate (DMP), butylbenzylphthalate (BBP), dibutylphthalate (DBP) and dioctylphthalate (DOP). Major human exposure to phthalates is believed to be from foods which have absorbed the chemical from their packaging or from the manufacturing process.

Polyaromatic Hydrocarbons (PAHs): raw foods should not normally contain high PAH levels. In areas remote from urban or industrial activity, the PAH levels found in unprocessed foods reflect the background contamination, which originates from long distance airborne transportation of contaminated particles and natural emissions from volcanoes and forest fires. The occurrence of PAHs in foods is influenced by the same physico-chemical characteristics that determine their absorption and distribution in man. PAHs are lipophilic and generally have very poor aqueous solubility. PAHs accumulate in lipid tissue in plants and animals. PAHs will not tend to accumulate in plant tissues with a high water content and limited transfer from the soil to root vegetables will occur. The transfer rate varies widely and is also influenced by soil characteristics, the plant involved and the presence of co-pollutants. PAHs strongly adsorb to the organic fraction of soils and do not penetrate deeply into most soils, therefore limiting both leaching to groundwater and availability for uptake by plants.

Some PAHs are semi volatile but most of them tend to be adsorb in organic particulate matter.

Heavier PAHs preferentially associate with particulate matter so atmospheric fallout is a principal route for contamination [17,18]. PAHs with 5 or more aromatic rings are found predominantly on particulates, (usually on small (< 2.5 μm) particles such as fly ash and soot). PAHs with 2 or 3 rings are almost entirely in the vapor phase, those with 4 rings being in an intermediate position.

Consequently, vegetables with large leaves, grazing cattle and poultry which may ingest particulate matter from soil are susceptible to contamination by PAHs adsorbed into particles.

The waxy surface of vegetables and fruits can concentrate low molecular mass PAHs mainly through surface adsorption. PAH concentrations are generally greater on plant surface (peel, outer leaves) than on internal tissue. Careful washing may remove up to 50% of total PAHs. Particle- bound PAHs are easily washed off the surface whereas those in the waxy layer are less efficiently removed, washing may alter the apparent high to low molecular mass PAH profile.

Parabens have been added to food for more than 50 years and their usage steadily increased to include more food categories, like soft drinks and frozen dairy products. Methyl and propyl are the most extensively used in foods, and the FDA has affirmed that they are Generally Recognized as Safe (GRAS) for direct addition to food at concentrations below 0.1%. A 30-fold increase in use of parabens was noted from 1960 to 1970. Parabens have also been used as preservatives in food [77].

PBDEs are a class of industrial chemicals that have been widely used in the manufacture of many materials found commonly in highly industrialized societies. They are currently used as effective flame retardant in plastics, electronics, automobiles, home furnishings, textiles, and in building materials. These compounds have been also used to leach contact material from food [78].

Exposure to PBDEs is mainly through house dust, indoor air, and direct contact with consumer products. Until more recently, food was not considered one of the most important ways in which people were exposed to PBDEs. But the fact is that the connection between PBDs and food has been revealed by different studies that report the presence of PBDEs in butter, fish and other foods, as well as other food containing animal fats (see occurrence paragraph).

Perfluorinated compounds (PFCs) are a class of man-made chemicals that are widely used in industrial and consumer products including protective coatings for fabrics and carpets, paper coatings, paints, cosmetics, and fire-fighting foams. As a consequence, PFCs are widespread in humans and animals. Potential sources of human exposure include indoor dust, diet, and drinking water. Human exposure to PFCs is of concern because studies have found that perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), the two most commonly studied PFCs, elicit hepatotoxicity, developmental toxicity, and immunotoxicity in laboratory animals.

The European Food Safety Authority set a tolerable daily intake (TDI) of 150 ng/kg bw/day for PFOS and 1500 ng/kg bw/day for PFOA based on contamination in the food chain.

Packaging and manufacturing [62] seems to be one of the most frequent ways to contaminate food with industrial endocrine-disruptor chemical compounds. A special EU regulation concerning the products, which can migrate from plastic containers to food, is established by Directive 2002/72/EC [19]. The directive applies to plastic materials and articles intended to come into contact with foodstuffs. Such materials and articles, and parts thereof, may be composed either of plastic material only or of several layers of plastic material or of different types of materials.

Plastic materials and articles coming into contact with foodstuffs may transfer toxic substances which present a risk to human health. All such substances are controlled at Community level by means of maximum limits on their migration to food, and are subject to very specific conditions of use, in the general interest of food safety.

Specific migration limits have been established for phthalates, Bisphenol A and alkylphenols. However, there are many more potential EDCs that could leach from food contact materials into foods (during storage, processing and packaging and when in use, i.e. if the food is heated inside the packaging). In the case of food packaging, not all substances that can migrate from the packaging into the food are even known about (http://www.foodbase.org.uk//admintools/reportdocuments/518-1 911_A03054_reaction_and_breakdown_products_final_report.pdf).

3.1. Analysis of industrial EDC chemicals in crops

In Table 2 we show an overview of different treatment methods and analytical methods for the determination of industrial chemicals in vegetable food and cereals. This includes the commodity category, sample matrix, compounds, pre-treatment, extraction, detection mode, chromatographic features, limits of detection and quantification and recoveries of all the work reviewed. Discussion of this table will be carried out looking at the preparation method and analytical method for the industrial chemical in general.

With regards to the analysis of PCBs, phthalates and BPA in crops, there are very few publications when compared to the analysis of other contaminants such as pesticides, more commonly found in crops as contaminants due to their use in agriculture.

3.1.1. Preparation method

A priority goal when analyzing BPA is to minimize background contamination through laboratory materials like solvents, Solid phase extraction (SPE) columns, glassware, plastic ware and other reagents and laboratory tools. In general, heat-treated glassware and solvent-washed materials are used as a precautionary measure to prevent background contamination [36]. For the analysis of BPA in fruits and vegetables, it is very important to control enzymatic degradation, Kang et al. [20] suggested that the prevention of BPA degradation by enzymes is possible by pH control, they found that the optimum pH for the analysis of BPA in fruits and vegetables is $pH \leq 3$. Sample pre-treatment usually comprises sample preconcentration and clean-up. Solvent extraction [20], Liquid–liquid extraction (LLE) [21] and Pressurized liquid extraction (PLE) [22] following a clean-up using SPE with polymeric HLB sorbent are the techniques used for the isolation of BPA in fruits, vegetables and cereals, as is shown in Table 2. Traditional techniques such as solvent extraction, LLE, and extraction in a Soxhlet apparatus are still the most widely used techniques for the isolation of PCBs from fruits and vegetables. For PCB analysis in fruit and vegetables, the samples are freeze-dried and mixed with a small amount of Na_2SO_4, to remove the water content for the subsequent Soxhlet extraction with an immiscible water solvent. Generally, the extracts containing PCBs are subject to extensive clean-up; a variety of different clean-up procedures have been reported, but most of them are essentially based on multilayer silica columns in combination with alumina, florisil or carbon columns. Some purification pre-treatments have also been described: sulphuric acid, silica gel KOH/H_2SO_4 and gel permeation chromatography (GPC). Zuccato et al. [23] have determined non-dioxin-like and dioxin-like PCBs in salmon, butter and cabbage. Cabbage samples were extracted with acetone, and the extracts purified with a combination of columns containing alternate layers of acidified and basic silica gel, separated by neutral gel and topped with a layer of anhydrous sodium sulphate. The extracts were then further purified and the non-ortho PCBs separated from the others by passage through an activated Florisil column. The limits of quantification obtained were of 5 ng/kg wet weigh in cabbage. In a similar way, Llobet et al. [24] have determined 11 PCB congeners from a variety of food commodities, including vegetables, fruits, cereals, fish and shellfish, meat, eggs, milk and oils and fats. In this case, Soxhlet extraction with toluene was used to extract the PCBs, and the clean-up and fractionation procedure was carried out as a multistep procedure involving adsorption chromatography, a multilayer

Table 2
Overview of representative analytical techniques used for the determination of selected groups of industrial chemicals EDCs in vegetables, fruits and cereals and animal origin products.

Commodity Categories	SAMPLE MATRIX	COMPOUNDS	PREPARATION METHOD			ANALYTICAL METHOD		LODs (µg/Kg or µg/L*)	LOQs (µg/Kg or µg/L*)	Recovery (%)	Ref
			PRETREATMENT	EXTRACTION		DETECTION MODE	LC OR GC COLUMN Mobile phase				
				Technic (Sorbent) Elution solvents	Clean-up/derivatization						
High wáter content	Orange, cucumber, lemon	**Multi-residue method for pesticides and PCBs** (Vinclozolin, malathion,carbendazim, prochloraz, Iprodion… and two PCBs)	Freezer before comminution	QuEChERS *Acetonitrile*	DSPE (PSA and anhydrous MgSO4)	LC-QLIT-MS/MS ESI (+), MRM GC-QqQ-MS/MS Use of analyte protectans	LC-QLIT-MS/MS: C18 *water(5mM ammonium formate)/ methanol (5Mm ammonium formate)* GC-QqQ-MS/MS: DB- 5 MS			86–120i,e	[64]
	Tomato	**Phthalates** (DMP,DEP,DEHP,DOP, DBP and BBP)	Frozen, liophilized, ground	Soxtec *n-Hexane*	SPE (Florisil) *n*-hexane:acetone	GC-Q-MS (EI), SIM	RTX-5 MS	3	10	90–110 i	[28]
	Grape, apple, banana	**Bisphenols** (BPA)	Homogenized, HCl addition (pH < 3), acetone and anhydrous sodium sulfate addition	Solvent extraction *Acetone*	SPE (Oasis HLB, Step-Pack Florisil)	LC-FD	RP-18 *AcN/water*	0,1		82–101 e	[20]
	Vegetables (tomatoes, lettuce…)	**PCB** (11)	Homogenized, liophilized, mixed with Na2SO4	Soxhlet *Toluene*	Adsorption chromatography, multilayer silica column, alumina columns and gel permeation columns (CioBeads SX3)	HRGC-HRMS (EI),MIM	DB-XLB			i	[24]
	Red grape	**Multi-residue method for pesticides and PCBs** (Vinclozolin, malathion,carbendazim, prochloraz, Iprodion… and two PCBs)	Freezer before comminution	QuEChERS *Acetonitrile*	DSPE (PSA and anhydrous $MgSO_4$)	LC-QLIT-MS/MS ESI (+), MRM	LC-QLIT-MS/MS: DB-5 MS *water (5 mM ammonium formate/ methanol (5 Mm ammonium formate)*			84–119i,e	[64]
	Cabbage	**PCBs** (18)		Solvent extraction *Acetone*	SPE (Silica gel and florisil)	HRGC-HRMS	DB-5 MS		0,005	60–110	[23]
	Potato, carrot.	**Bisphenols** (BPA)	Homogenized, HCl addition (pH < 3), acetone and anhydrous sodium sulfate addition	Solvent extraction *Acetone*	SPE (Oasis HLB, Step-Pack Florisil)	LC-FD	RP-18 *AcN/Water*	0,1		89–97 e	[20]
High oil content	Vegetable oil	**Phthalates** (DEHP, DBP, BBP and DINP)	Mixed with *n*-hexane	LLE *n-hexane/AcN*	LLE can:NaCl/n-hexane SPE (Florisil and Bondesil PSA)	GC–MS (EI)	DB-5 MS	10–200*		101–131 e,i	[25]
	Vegetable oil	**Phthalates** (DEHP, DBP, DOP and DEP)		LLE *Ethanol*		GC–MS ITD	DB-5	0,5–44*		89–102 e	[26]
	Olive oil	**Phthalates** (DMP,DEP,DEHP,DOP, DBP and BBP)	Diluted with GPC mobile phase (Ciclohexane:dichloromethane)		GPC Ciclohexane:dichloromethane	GC-IT-MS/MS (EI) and (CI+)	5-MS	(EI): 0,1–148* (CI +) : 0,1–156*	(EI): 0,2–182* (CI+): 0,4–195*	84–101/ 66–108 i,e	[27]
	Vegetable oil (olive, rap, peanut, corn, sunflower)	**Analysis of selected compounds** (Di-tertbutyl-4 hydroxymethylphenol, 2-NL,OPP and BHA)		LLE *AcN-hexane*		LC-TOF-MS (ESI -)	XDB-C18 *AcN/water*	1–30*		84–102 e	[21]
	Vegetable oil	**PCDD/Fs** (17) and **dl-PCBs** (12)	Saken for homogenization	LLE *n-hexane+H2SO4*	Automated clean up: Power-Prep TM System (silica, basic alumina, PX-21 carbon absorbents)	GC-IT-MS/MS (EI+), AGC	DB-5 MS	0,00004–0,0002* 0,00008–0,0006*		i	[65]
	Vegetable oil (olive and sunflower)	**PCB** (11)	Disolved in *n*-hexane		Adsorption chromatography, multilayer silica column, alumina columns and gel permeation columns (CioBeads SX3)	HRGC-HRMS (EI),MIM	DB-XLB			i	[24]
High starch and/or protein content and low	Wheat flour	**Multi-residue method for pesticides and PCBs** (Vinclozolin, malathion,carbendazim,	Employed directly for extraction Added cold water	QuEChERS *Acetonitrile*	DSPE (PSA and anhydrous $MgSO_4$)	LC-QLIT-MS/MS ESI(+), MRM GC-QqQ-MS/MS Use of analyte protectans	LC-QLIT-MS/MS: DB-5 MS *water (5 mM ammonium formate/ methanol (5 Mm*			69–112i,e	[64]

Table 2 (*continued*)

Commodity Categories	SAMPLE MATRIX	COMPOUNDS	PREPARATION METHOD			ANALYTICAL METHOD		LODs (μg/Kg or μg/L*)	LOQs (μg/Kg or μg/L*)	Recovery (%)	Ref
			PRETREATMENT	EXTRACTION		DETECTION MODE	LC OR GC COLUMN Mobile phase				
				Technic (Sorbent) Elution solvents	Clean-up/derivatization						
	water and fat content		prochloraz, Iprodion… and two PCBs)				*ammonium formate)* GC-QqQ-MS/MS: C18				
		Cereal	**APEOs** (4-NPs and 4-t-BP)	Ground, mixed with Hydromatrix	PLE *Methanol*	SPE (Oasis HLB) AcN:ethyl acetate	LC-Q-MS (ESI-), SIM	C18 *MeOH/0,0025 M ammonium formate buffer (pH3) with formic acid*	3–12		81–104e	[22]
		Cereal	**Chlorophenols** (DCP, TCP and PCP)	Ground, mixed with Hydromatrix	PLE *Methanol*	SPE (Oasis HLB) AcN:ethyl acetate	LC-Q-MS (ESI-), SIM	C18 *MeOH/0,0025 M ammonium formate buffer (pH3) with formic acid*	3–13		81–104e	[22]
		Cereal	**Bisphenols** (BPA)	Ground, mixed with Hydromatrix	PLE *Methanol*	SPE (Oasis HLB) AcN:ethyl acetate	LC-Q-MS (ESI-), SIM	C18 *MeOH/0,0025 M ammonium formate buffer (pH3) with formic acid*	43		81–104e	[22]
		Cereal	**4- tert-butylbenzoic acid** (BBA)	Ground, mixed with Hydromatrix	PLE *Methanol*	SPE (Oasis HLB) AcN:ethyl acetate	LC-Q-MS (ESI-), SIM	C18 *MeOH/0,0025 M ammonium formate buffer (pH3) with formic acid*	4		81–104e	[22]
		Cereals (bread, pasta and rice)	**PCB** (11)	Homogenized, liophilized, mixed with Na_2SO_4	Soxhlet *Toluene*	Adsorption chromatography, multilayer silica column, alumina columns and gel permeation columns (CioBeads SX3)	HRGC-HRMS (EI),MIM	DB-XLB			i	[24]
Meet and seefood	Crustacean (shrimp), mollusc (octopus), fish (anchovy, tuna, hake..)	APEOs 17 (Ops, NPs and ethoxylates)	**Lipid extraction, *n*-hexane disolved**	LLE *n*-hexane/I:NaOH LLE I:NaOH/isooctane	Derivatization (TFAA)	GC-IT-Ms/Ms (EI), SIM	DB-XLB			62–92 I,e	[43]
	Bilie fish (sea bass)	APEOs (4-NP and 4-t-OP) and OH-PAHs (1-napthol, 9-fluorenol, 9-phenantrol, 1-pyrenol)	**Hidrolized with 0,4 M acetic acid/sodium acetate buffer pH 5.0**	LLE Ethyl-acetate	Derivatization (BSTFA)	GC–MS (EI), SIM	HP-5 MS			e	[31]
	Bilie fish (sea bass)	Analysis of organochlorine compounds (14 PCBs, DDTs, HCB)	**Liophilised**	Soxhlet N-hexane:dichloromethane	LLE *n*-hexane/H_2SO_4 and Solid-liquid adsorption chromatography	GC-ECD	CP-Sil 5 CB			e	[31]
	Fish (rainbow trout)	APEOs (4-t-OP) and BPA	**Frozen with liquid nitrogen, crushed**	MAE Dichloromethane:methanol	SPE (Sep-Pak NH_2)	LC-Q-MS (APCI -), SIM	XDB-C18 MeOH:water/MeOH	APEOs: 10 BPA: 50		≥ 95 I,e	[39]
	Crustacean (shrimp, lobster),fish (hake, mullet, angler..)	APEOs (OP, NP and OPEs)	**Fat extraction, n-hexane disolved**	LLE *n*-hexane/I:NaOH LLE I:NaOH/isooctane		GC–MS SIM	Rtx-5	0,5–8,1*		I,e	[63]
	Fish (salmon)	PCBs (18)		Solvent extraction Hydrochloric acid and dichloromethane:n-hexane	SPE (Silica gel and Florisil)	HRGC-HRMS	DB-5 MS		0,005	60–110	[23]
	Fish (sea bass)	PCBs (102)	**Liophilised**	Soxhlet N-hexane:acetone	SPE (Extrelut with H_2SO_4 and silica gel column),	HRGC-HRMS (EI), SIM	SGE HT8			≥ 50 i	[32]
	Crustaceans (edible crab, lobster..)	PCBs (18) and PBDEs (9)	**Muscle dissected, freeze-dried, ground**	Soxhelt Toluene-cyclohexane	Sulphonation (H_2SO_4) for fat removal, multil-layed adsorption chromatography (silica gel) Hexane, SPE (Florisil) Heptanes and SPE (Carbopack B) for separation	HRGC-HRMS	DB-5 and /or HT8-PCB	0,001–0,02		80–110 i	[33]
	Crustaceans (edible crab, lobster..)	PCDD/Fs (27)	**Muscle dissected, freeze-dried, ground**	Soxhelt Toluene-cyclohexane	Sulphonation(H_2SO_4) for fat removal, multil-layed adsorption chromatography (silica gel) Hexane, separation SPE (Florisil) Toluene for separation	HRGC-HRMS	DB-5 MS	0,001–0,02		80–110 i	[33]
	Fish, crustacean(mullet, crab…)	PCDD/Fs (15) and PCBs (12)	**Homogenized**	ASE Toluene	SPE (Extrelut with H_2SO_4, silica, alumina, carbon)	HRGC-HRMS (EI)	DB-5 MS and Rtx 200 (PCDD/Fs) DB-5 MS and SPB-Octyl (PCBs)			i	[29]

	Fish (tuna)	PCDFs (17) and dl-PCBs (16)	**Blended with anhydrous sodium sulphate**	Soxhlet Methylene chloride:hexane		HRGC-HRMS (EI)					[53]
	Fish and crustacean (sardine, tuna, shrimp..)	PCDD/Fs (17) and PCBs (11)	**Homogenized, blended, liophilized,mixed with Na_2SO_4**	Soxhlet Hexane-dichloromethane	Adsorption chromatography, multilayer silica column, alumina columns and gel permeation column (BioBeads SX3)	HRGC-HRMS (EI),MIM	DB-XLB			i	[66]
	Mussel and Fish (hake, sardines)	PCB (11)	**Homogenized, blended, liophilized,mixed with Na_2SO_4**	Soxhlet Hexane-dichloromethane	Adsorption chromatography, multilayer silica column, alumina columns and gel permeation columns (BioBeads SX3)	HRGC-HRMS (EI),MIM	DB-XLB			i	[24]
	Fish, crustacean(mullet, crab…)	PAHs (16)	**Homogenized**	ASE Toluene	SPE (silica gel)	HRGC-HRMS (EI), SIM	Rtx-5			i	[29]
	Meat (pork, rabbit, duck, chicken..) and fish	APEOs (OP and NP) and BPA	**Homogenized, mixed with celite**	ASE Acetone	SPE (amino-propyl)	LC-MS/MS (ESI -), MRM	C18 MeOH/water (0,1%ammonia)	APEOs: 0,05–0,1 BPA: 0,3	APEOs: 0,2–0,4 BPA:1	89–10 I, e	[38]
	Liver and fat of hens	PCDD/Fs and PCBs(dioxins)	**Fat extraction, mixed with MeOH:Chloroform, centrifuged**	GPC for fat removed	SPE (alumina), HPLC (Porous arbónized carbon)	HRGC-HRMS					[35]
	Liver and fat of hens	PCBs (7)	**Fat extraction, mixed with MeOH:Chloroform, centrifuged**		On-line clean-up over a silica HPLC column	(LVI) GC–MS SIM					[35]
	Meat (beef, chicken)	PCDD/Fs (15) and PCBs (12)	**Homogenized**	ASE Toluene	SPE (Extrelut, silica, alumina, arbón)	HRGC-HRMS (EI)	DB-5 MS and Rtx 200 (PCDD/Fs) DB-5 MS and SPB-Octyl (PCBs)			i	[29]
	Meat (beef, chicken)	PAHs (16)	**Homogenized**	ASE Toluene	SPE (silica gel)	HRGC-HRMS (EI), SIM	Rtx-5			i	[29]
	Meat (beff, chicken, pork, lamb..)	PCB (11)	**Homogenized, blended, liophilized, mixed with Na_2SO_4**	Soxhlet Hexane-dichloromethane	Adsorption chromatography, multilayer silica column, alumina columns and gel permeation columns (CioBeads SX3)	HRGC-HRMS (EI), SIM	DB-XLB			i	[24]
	Meat (Chicken and pork)	PCBs				GCxGC-μEDD	DB-XLB /LC-50			i	[67]
Milk and milk products	Milk	Phthalates (DMP,DEP,DEHP,DOP, DBP and BBP)	**NaCl addition, heated**	HS-SPME		GC-Q-MS SIM	DB-5	0,31–3,3*		82–104 i	[55]
	Milk	Phthalates (DMP, DEP, DBP, BBP and DEHP)	**Water and methanol addition, sonicated**	SPE (C18) Ethyl acetate	SPE (Florisil)	GC-Q-MS (EI), SIM	HP 5 MS	0,06–0,12*		81–100 i, s	[40]
	Milk and ice cream	Phthalates (DEHP, DBP, BBP and DINP)	**Water,ethanol and sodium oxalate addition**	LLE Ether:n-hexane	SPE (Florisil , Bondesil PSA)	GC–MS (EI)	DB 5 MS	2–60*		98–125 e,i	[25]
	Human milk	PHENOLS (BPA, OPP, 2,4-dichlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol and BP-3)	**Incubated, Me OH addition, centrifugated and formic acid addition.**	On-line SPE Methanol:water		LC-QLIT-MS/MS (APPI -)	XDB-C18 Water/MeOH	0,1–0,4*		90–107 i	[34]
	Milk	PHENOLS (BPA, NP and BPA diglycidyl ether)	**Methanol addition, sonicated, water addition.**	SPE (C18) Ethyl acetate	SPE (Florisil)	GC-Q-MS (EI), SIM	HP 5 MS	0,15–0,36*		73–119 i, s	[40]
	Milk condensed	Bisphenols (BPA)	**Water dilution, water-methanol addition**	SPE (C18) Methanol:water		LC-Q-MS (ESI-), SIM	100-RP18 Water/MeOH	1*	5*	83 i	[41]
	Human milk	Parabens (methyl-, ethyl-, propyl-, butyl- and benzyl parabens)	**MeOH addition, centrifuged, formic acid addition**	On-line SPE Methanol:water		LC-QLIT-MS/MS (APPI -)	XDB-C18 Water/MeOH	0,1*		90–106 i	[34]
	Milk	PCB (11)	**Homogenized,liophilized, mixed with Na_2SO_4**	Soxhlet Toluene	Adsorption chromatography, multilayer silica column, alumina columns and gel permeation columns (CioBeads SX3)	HRGC-HRMS (EI), MIM	DB-XLB			i	[24]
	Milk	PCDDs, PCDFs and dl-PCBs		LLE N-hexane:diethyl ether	SPE (Silica column with H2SO4)	DR CALUX bioassay (Screening)					[68]
	Milk	PCDDs, PCDFs and dl-PCBs	**Water dilution, sodium oxalate addition**	LLE MeOH:diethyl ether: n-hexane	SPE (Extrelut with H_2SO_4), Power-Prep TM System (silica, alumina, graphitic arbon)	HRGC-HRMS (confirmation) SIM					[68]
	Cheese and Yogourt	PCB (11)	**Homogenized, blended, liophilized,mixed with Na_2SO_4**	Soxhlet Toluene	Adsorption chromatography, multilayer silica column, alumina columns and gel permeation columns (CioBeads SX3)	HRGC-HRMS (EI), MIM	DB-XLB			i	[24]

Table 2 (*continued*)

Commodity Categories	SAMPLE MATRIX	COMPOUNDS	PREPARATION METHOD			ANALYTICAL METHOD		LODs (μg/Kg or μg/L*)	LOQs (μg/Kg or μg/L*)	Recovery (%)	Ref
			PRETREATMENT	EXTRACTION		DETECTION MODE	LC OR GC COLUMN Mobile phase				
				Technic (Sorbent) Elution solvents	Clean-up/derivatization						
	Cheese	Phthalates (DEHP, DBP, BBP and DINP)	**Ground, mixed with ACN, centrifuged**	Solvent extraction AcN	LLE AcN/hexane SPE (Florisil , Bondesil PSA)	GC–MS (EI)	DB 5 MS	4–60		e,i	[25]
Eggs	Eggs	PCDD/Fs and PCBs(dioxins)	**Fat extraction , mixed with MeOH:Chloroform, centrifuged**	GPC for fat removed	SPE (alumina), HPLC (Porous graphitized carbon)	HRGC-HRMS					[35]
	Eggs	PCBs (7)	**Fat extraction , mixed with MeOH:Chloroform, centrifuged**		On-line clean-up over a silica HPLC column	(LVI) GC–MS SIM					[35]
	Eggs	Analysis of organochlorine pesticides and PCBs (HCH, HCB, Heptachlor, Aldrin, Dieldrin, Chlordane,Endrin, Methoxychlor, Nitrofen, DDT and eight PCBs	**Fat extracted: hexane and acetone**	Solvent extraction Hexane	SPE (Alumina) hexane	Pesticides:GC-ECD Two GC columns and two ECD detectors PCBs:GC-ECD GC-IT-MS/MS	HT-8 DB1701		10*	i, s	[69]
High sugar and low water content	Honey	Bisphenols (BPA and BPF)	**Dissolved in water, and hydrocloric acid addition**	SPE (GL-Pak PLS 2) Methanol		LC-FL LC/MS (confirm) (ESI-)	C18 Water/CAN Water (0,1%acetic) acid/ACN			98–104	[59]
Fat from food of animal origin	Pork fat	PCB (28)	**Homogenized, blended, liophilized, ,mixed with Na_2SO_4**	PLE N-pentane	SPE (silica gel and H2SO4)	GC-μEDD	DB17 / HP5-HT5			i, e	[70]
	Butter	Phthalates (DEHP, DBP, BBP and DINP)	**Melted, solvent extraction (acetone), filtered, evaporated and NaCl addition**	LLE N-hexane /ethyl acetate LLE N-hexane/ACN	SPE (Florisil , Bondesil PSA)	GC–MS (EI)	DB 5 MS	10–280		92–118 e	[25]
	Butter	PCBs (18)		Solvent extraction H2SO4 and dichloromethane: n-hexane	SPE (Silica gel and Florisil)	HRGC-HRMS	DB-5 MS		0,05	60–110	[23]
	Butter	PCDD/Fs (15) and PCBs (12)	**Homogenized**	Sonication N-hexane	SPE (Extrelut, silica, alumina, carbon)	HRGC-HRMS (EI)	DB-5 MS and Rtx 200 (PCDD/Fs) DB-5 MS and SPB-Octyl (PCBs)			i	[29]
	Butter	PAHs (16)	**Homogenized**	Sonication N-hexane	SPE (silica gel)	HRGC-HRMS (EI), SIM	Rtx-5			i	[29]

i, internal calibration.
e, external calibration.
s, with surrogate standard.
* indicates result in μg/L

silica column (from top to bottom: sodium sulfate, silica, silica-sulphuric acid, silica, silica-potassium hydroxide, silica) alumina columns, and gel permeation columns (BioBeads SX3).

It is very important when analyzing phthalates to reduce sample treatment to the minimal steps in order to avoid, or minimize, phthalate contamination through laboratory materials like tubing, pipette tips, septa, solvents, etc. In general, a blank sample is prepared under the same conditions simultaneous with the test sample and reflects the contamination levels present in the laboratory, reagents and apparatus. The contamination contribution values reflected in the blank sample is averaged and subtracted from the detected values of the plasticizers in the real sample [25,26].

Phthalates are present even at 40% (w/w) in soft Poly Vinyl Chlorine (PVC), show high solubility in fat and alcohol whereas their solubility in water is restricted. Since phthalates do not form stable bonds with polymers to which they are added, they tend to migrate in contact materials, especially in the case of oily and fatty foodstuffs. Consequently, the most frequently used ester, DEHP, became a ubiquitous pollutant in the environment and, particularly, in foods. In general, since phthalates are lipophilic, these compounds tend to be distributed mostly in fatty food and this can cause the presence of remarkable amounts of these contaminants in vegetable oils. Several methods for the determination of phthalates in fatty matrices have been published. Some procedures use LLE or Solvent Extraction (SE) followed by clean-up using adsorption column chromatography on Florisil or alumina [25], but most of the clean-up steps currently applied are based on GPC [27]. This methodology is relatively effective at removing fats and oils and is applicable to a wide range of analytes such as pesticides, PAHs and phthalates. Cavaliere et al. [27] have developed a method for the determination of phthalates in olive oil by GC–MS/MS analysis following a preliminary GPC clean-up step. The high specificity and signal-to-noise ratio obtained by matching GPC techniques with MS/MS allowed the authors to exclude any preliminary LLE prior to GPC purification, or a further SPE clean-up step currently applied in the extraction of phthalates in fat matrices. Other authors have extracted phthalates from vegetable oil using successive LLE with ethanol [26] and LLE with hexane/acetonitrile in combination with Florisil and Bondesil PSA dual layer columns as SPE sorbents [25] followed by GC–MS in SIM mode.

Phthalates, DEHP in particular, are present in quite high concentrations in sewage sludge and they could accumulate in the soil after land application, and therefore be absorbed by plants. For this reason, Sablayrolle et al. [28] developed and validated a method for the trace determination of six phthalates in sludge and tomato. For this purpose, the authors lyophilize the sample prior to Soxhlet extraction, using a Soxtec apparatus, with n-hexane and extract purification with SPE using Florisil sorbent followed by identification and quantification by Gas Chromatography mass spectrometry (GC–MS) in Selected Ion Monitoring (SIM) mode.

3.1.1.1. Analytical method. The analytical methodology employed in the analysis of industrial chemicals with endocrine disruption action in crops, is based mainly on Liquid Chromatography mass spectrometry (LC–MS) and GC–MS as is showed in Table 2.

Due to their physico-chemical properties, the determination of phthalates, polychlorinated dibenzo-p-dioxins, dibenzofurans and polychlorinated biphenyls is mainly performed by GC–MS.

In general, non-polar analytical columns have been employed in most of the methods with a stationary phase composition of 5% phenyl-95% dimethylpolysiloxane.

Concerning the ionization mode proposed by the authors, electron ionization is the choice, and the acquisition mode is MS/MS.

Zuccato et al. [23] have determined non-dioxin-like and dioxin-like PCBs in cabbage by High Resolution gas chromatography-High Resolution mass spectrometry (HRGC-HRMS). The PCB congeners were IUPAC numbers 28, 52, 101, 118, 153, 138 and 180 (the most abundant, also called the "EC7" or the "seven indicators"), 105, 114, 123, 156, 157, 167, 189 ("dioxin-like" mono-ortho PCBs), and 81, 77, 126, 169 ("dioxin-like" non-ortho PCBs). The congeners were all determined by high-resolution gas chromatography–high-resolution mass spectrometry. Briefly, stable 13C-labeled analogs of the WHO 12 PCBs were added to homogenized aliquots of each test sample. The average molecular ion response, calculated as the average response of the standards of each group, was used to quantify the PCBs of each chlorinated class and the total PCBs.

Llobet et al. [24] have determined 11 PCB congeners. Polychlorinated biphenyl (PCB) concentrations were determined for 108 samples including vegetables, fruits, cereals and oils. Levels of 11 PCB congeners (IUPAC 28, 52, 77, 101, 105, 118, 126, 138, 153, 169, and 180) were determined by high-resolution gas chromatography–high-resolution mass spectrometry.

Although processed food was not reviewed for this work, some references of vegetable oils for determination of phathalates [25–27] have been considered and commented on in this section. Plasticiers in Japanese retail foods were determined by gas chromatography/mass spectrometry (GC/MS) (SIM) [25]. The plasticisers tested were as follows: dibutyl phthalate, butylbenzyl phthalate, di(2-ethylhexyl)phthalate (DEHP), diisonyl phthalate, di(2-ethylhexyl) adipate, diisonyl adipate (DINA), dialkyl adipate, dibutyl sebacate, o-aceteyl tributil citrate (ATBC) and diacetyl-lauronyl glycerol (DALG).

In Cavalier et al. [27], a gas chromatography–tandem mass spectrometry (GC–MS/MS) method for the detection of six phthalates (dimethyl phthalate (DMP), diethyl phthalate (DEP), di-*n*-butyl phthalate (DBP), butylbenzyl phthalate (BBP), di-2-ethylhexyl phthalate (DEHP) and di-*n*-octyl phthalate (DOP)) in olive oil was developed. Two ionization methodologies, electron impact (EI) and isobutane-chemical ionization (CI) were compared in MS/MS mode to achieve better analytical performance.

Sablayrolles et al. [28] have developed a method for the determination of phthalates in tomato plants. Six phthalates were studied simultaneously: dimethylphthalate, diethylphthalate, di-*n*-butylphthalate, nbutylbenzylphthalate, di-2-ethyl-hexyl phthalate (DEHP) and di-*n*-octylphthalate. Precise, sensitive and selective identification and analyte quantification was carried out using GC–MS in the single-ion-monitoring mode. This protocol allows analytes with concentrations as low as 10 g/kg dry matter (DM) to be determined from small (1–2 g DM) samples. This analytical method has been applied to a phthalate transfer study for agricultural recycling of sludges.

Results from this study show that in terms of phthalate transfer; only DEHP is important. However, even for this phthalate, transfer into the tomato plant remains very low.

The determination of BPA in food is mainly carried out by Liquid chromatography-fluorescence detector (LC-FD), LC–MS and GC–MS. LC offers the advantage of simplicity over GC for which a derivatization step is generally necessary, while the latter provides higher peak resolution.

The determination of phenolic compounds is mainly performed by an LC–MS based method using electrospray in negative mode as the ionization system.

Xiu-Qin et al. [21] developed a method to evaluate a group of phenols in edible vegetable oils. The analytical determination for this group of antioxidants and preservatives was performed by a LC–MS technique, with a time-of-flight analyzer; these kinds of food matrices are one of the most complex due to the presence of numerous interferences that show up in full-scan mode. For this reason, high performance liquid chromatography time of flight

mass spectrometry (HPLC/TOF-MS) parameters must be optimized to investigate the fragmentation of the analytes studied. In this study, the fragmentor voltage was optimized in order to obtain additional information from characteristic fragments of the target compounds. The method is fully validated and is applied in routine analysis for the screening and quantitation of antioxidants and preservative endocrine-disruptor compounds in edible vegetable oils. When there is the possibility of isobaric interferences with these kinds of samples, due to the complexity of the matrix, the use of mass spectrometric techniques with high selectivity is absolutely essential. In this sense, the selectivity of HPLC/TOF-MS relies on the resolving power of the instrument on the m/z axis, which enables discrimination between the target species and the isobaric interferences within 0.01 Da of mass difference.

3.2. Analysis of industrial EDC chemicals in products of animal origin

3.2.1. Preparation method

Butter, fats and oils are generally assumed to be homogeneous, and normally do not require extensive extraction procedures. Aliquots of such samples can be dissolved in n-hexane or petroleum ether to the desired concentration [29].

Meat and fish products having a lipid content of ca. 10 wt% or lower are initially blended and homogenized. Next, a representative test sample is ground with anhydrous sodium sulphate, until a free-flowing powder is obtained or lyophilized [24,30–33].

Milk can either be freeze-dried [24] or chemically-dried with anhydrous sodium sulphate, or subjected to an LLE procedure consisting of mixing with sodium oxalate and ethanol or methanol, followed by (repeated) extraction steps with a combination of organic solvents [25]. Other authors would initially separate fat by centrifugation [34].

In the analysis of eggs, normally the egg yolk and white are separated by fat extraction from the egg yolk [35].

It is common to determine BPA with other phenols or alkylphenolic compounds in food.

Human exposure to BPA and its derivatives occurs primarily via food in contact with BPA-containing materials, but food may also contain BPA as a result of diffuse environmental pollution and direct uptake by animals via food or air and potential bioaccumulation and transfer through the food web.

Many analytical methods have been developed for determining alkylphenols and BPA residues in products of animal origin. Classical approaches for the extraction of these compounds in solid matrices are mainly based on Soxhlet extraction [36], steam distillation [37] or solvent extraction [31]. Methanol, acetonitrile, ethyl acetate, acetone, n-hexane or mixtures of more than one of them are the typical solvents. Over the past decade, a lot of extraction techniques have been developed to isolate these analytes in solid samples, to reduce the organic solvent consumption and to increase the speed of the process. Accelerated solvent extraction (ASE) is one of the main extraction techniques for these compounds in solid matrices. The solvents chosen for ASE can be the same as those used in Soxhlet or solvent extraction. Microwave-assisted extraction (MAE) is also an efficient extraction technique for solid samples. MAE has a disadvantage compared to ASE, the need for sample centrifugation and filtration, which can have critical effects on analytical accuracy. However, MAE offers the ability to extract several samples simultaneously, while, in ASE, samples are run one at time. Due to the complexity of matrices, purification of the extracts after extraction has become crucial in order to obtain the maximum sensitivity in the subsequent detection of analytes. For the simultaneous determination of alkylphenols (NP and OP) and BPA in different meats, Shao et al. [38] developed and applied a method based on accelerated solvent extraction, with a subsequent clean-up step using amino-propyl solid-phase extraction cartridges and LC-ESI-MS/MS. Pedersen et al. [39] developed a method based on MAE followed by SPE using amino-propyl cartridges and LC-APCI-MS for the simultaneous determination of 4-t-OP and BPA in fish tissue. The recoveries of these compounds in both methods were over 89%.

LLE and SPE are frequently used for both extraction of alkylphenolic compounds and BPA from liquid samples, such as milk [40,41], and clean-up procedures. Several sorbents have been utilized. Non-polar reverse-phase (RP) sorbents with a silica base were the first used in SPE for these compounds. Among these sorbents (C_2, C_8 and C_{18}), C_{18} was the most acceptable. Despite evidence than C_{18} sorbents present good recoveries [40,41], they also show low breakthrough volumes for NP, OP and others alkylphenols [42]. The hydrophobic polymeric sorbents, such as Isolute ENV (International Sorbent Technology Ltd., Hengoed, UK) or LIChrolut EN (Merck, Darmstadt, Germany) have obtained similar recoveries to those of C_{18} silica-based materials [42]. Nevertheless, these sorbents are the most suitable, due to their broad range of physico-chemical characteristics and their greater chemical stability. Moreover, the breakthrough volumes are greater than those obtained with C_{18} sorbents. The divinylbenzene/N-vinylpyrrolidone copolymer (OASIS HLB from Waters) has been the most used sorbent to date to extract BPA. However, NP is not adequately recovered from Oasis HLB cartridges [42]. SPE has been developed in the off-line and on-line modes, although the on-line approach is preferred due to its advantages (e.g. greater sensitivity and fewer requirements for manipulation of samples). Ye et al. developed a sensitive method, using a unique on-line SPE-LC-MS/MS system with a peak focusing feature, to measure BPA, ortho-phenylphenol (OPP), 2,5-dichlorophenol and others phenols in milk [34]. Recoveries were above 84% for all analytes and the detection limits for most of the analytes were below 1 ng/mL in a small amount of samples (0.1 mL) and meant minimum sample preparation. The method showed itself to be rugged as well as labor- and cost-effective.

GC–MS provides higher resolution and lower detection limits than LC-MS for the determination of BPA and alkylphenolic compounds in food, although the need for a derivatization step makes the GC-based methods labor-intensive and introduces new potential sources of errors, mainly due to contamination. On the other hand, since the presence of lipids can significantly reduce the analytical performance of GC, extensive clean-up is required for fatty food, such as fish or meat. The inclusion of a derivatization step leads to sharper peaks and, consequently, better separation from other analytes and co-extracted matrix components, in addition to higher sensitivity (sub ng/g levels). Silylation and acetylation have been the most used derivatization procedures, which are usually carried out by adding 100–200 μL of the corresponding reagent to the dried extract and allowing the mixture to stand for 30–60 min at room temperature or at 65–80 °C. Silylation of the active hydrogens of these compounds is mainly made using bis(trimethylsilyl)trifluoroacetamide (BSTFA) [31]. The addition of 1% trimetylchlorosilane (TMCS) favours the formation of a single derivative, since the reaction of BSTFA with analytes having different hydroxyl groups, such as BPA, can generate several derivatives, thus reducing the sensitivity and selectivity of the analysis. Silylation of BPA and other phenols has also been carried out using methyl-trimethylsilyltrifluoroacetamide (MSTFA) and MTBSTFA. Acetylation of the hydroxyl groups of alkylphenolic compounds and BPA with acetic anhydride or TFAA [43] is the other common procedure to obtain derivatives of these compounds for GC–MS.

The extraction of perfluorinated compounds from solid food samples such as salmon, beef or pork is commonly performed by solid-phase extraction after alkaline digestion (to destroy lipids and proteins) and sonication followed by acidification [79].

3.2.2. Analytical method

Analytical determination of most of the industrial chemicals reviewed in this paper has been performed by GC (see Table 2), the groups of industrial chemicals analyzed in animal-origin products by GC are mainly PCBs, PBDEs, PCDD/Fs, parabens, phtalates and PAHs.

Analytes have been separated mainly using non-polar columns (CP Sil 5, DB-5MS, Rtx-5ms, ZB5, HP-5MS) or mid-polar columns (DB 17) for the general analysis of PCBs, PBDEs, PCDD/Fs, Phtalates and PAHs. Non-polar columns for PCB confirmation (DB-XLB, SPB-octyl, SGE-HT8) and polar columns for PCDD/F confirmation (DB 225, Rtx 200) have been used.

Traditional detectors have been employed by a minority of authors: Fernandes et al. [31] used Gas chromatography electron capture detector (GC-ECD) for the determination of organochlorinated compounds, which included the determination of 14 PCBs in fish samples. The quantitation was performed using and external calibration mixture of the selected congeners. Sporring et al. [70] developed a method for the determination of 28 PCB congeners in pork fat using a GC-μECD system. Haglund et al. [67] developed a GCxGC-μECD for the determination of dibenzo-p-dioxins and dibenzofurans in chicken and pork. In this paper, it has been demonstrated that GCxGC offers both exceptional separation power (peak capacity) and low limits of detection (LODs) (0.5 pg), combined with μECD, sufficient selectivity and sensitivity can be achieved to allow PCDD/F measurements at levels close to, or even below, the EU maximum and action levels. In this paper, an interlaboratory test was performed in order to compare results obtained with GCxGC-μECD and results obtained with GC–MS, results were comparable for the selected compounds, although a slight tendency towards overestimation was observed for GCxGC-μECD.

Mass spectrometry is the detection system of choice for most authors when PCBs, PBDEs, PCDD/Fs, Phatalates and PAHs are analyzed. Electron-impact ionization is the ionization technique preferred for analysis of these compounds in animal-origin products, and SIM mode is the acquisition mode of choice. Casajuana et al. [40] developed a method for the determination of phatalate esthers, bisphenol A, bisphenol A diglycidyl ether and nonylphenol in milk. Three diagnostics ions were selected for the correct identification of compounds, and the limits of detection reported were between 0.06 and 0.36 μg/Kg.

Liquid chromatography offers the advantage of simplicity over GC (for which a derivatization step is necessary). LC coupled to fluorescence detection (FD) has been applied for the analysis of alkylphenolic compounds and BPA in very different food matrices [44]. Other spectrometric detection systems (e.g. UV or electrochemical detection (ED)) have also been employed for the same purpose, but they provided less selectivity and lower sensitivity than FD systems, which showed results than can be compared with those obtained with simple MS detectors. Nevertheless, these optical detectors are becoming out of date when faced with MS detection, mainly due to the lack of specificity in analysis.

Alkylphenolic compounds and BPA have been analyzed by a wide range of chromatographic analysis techniques, ranging from GC–MS over normal-phase liquid chromatography with fluorescence detection to reverse-phase liquid chromatography with MS or MS/MS detection. Some of the analytical methods described for the determination of these compounds and other EDCs in products of animal origin are listed in Table 2.

Over the past decade, LC–MS technologies have surged to become an important tool for the identification and the quantification of BPA and alkylphenolic compounds. ESI and APCI interfaces are the most widely employed for the LC–MS analysis of these compounds. ESI is more frequently used than APCI because it generally provides better sensitivity in negative-ionization (NI) mode; however, APCI provides additional structure information. In positive-ion (PI) mode the sensitivity of APCI is almost as good as ESI and is generally less sensitive to matrix interferences. For all alkylphenolic compounds and BPA, the NI mode is employed almost exclusively. When using ESI in positive mode, the APEOs almost solely form sodium adducts and these are often chosen as the parent ion. Unfortunately, there is still rather poor sensitivity towards alkylphenol mono- and di-ethoxylates, which is often improved by measuring $[M+NH_4]^+$ instead $[M+Na]^+$.

When working with reverse-phase separation, it is not possible to separate APEOs with different numbers of ethoxylate units, but separation between OP and NP, as well as their ethoxylates and carboxylates, is obtained. Normal-phase chromatography, on the other hand, can separate APEOs as a function of their number of ethoxylate units, but cannot separate the different alkylphenol species.

Phenols, parabens and APEOs are typically determined in animal-origin products by LC, but there are some publications in which determinations are performed by GC, such BPA, and noylphenol [40] with very good limits of detection.

APEOs has been analyzed in crustaceans and Bilie fish by GC–MS after derivatization using TFAA [43] and BSTFA [31] as the derivatizing agent.

As we have commented on before, the most common analytical technique for the analysis of phenols, parabens and APEO compounds is liquid chromatography (see Table 2).

The analytical columns employed for the separation of APEOs, bisphenol A and parabens are generally C18 columns with a mobile phase composition of methanol:water.

Mass spectrometry is the detection system of choice and different ionization sources have been employed for the analysis of these compounds in products of animal origin, APPI in negative mode has been employed for the analysis of Bisphenol A and parabens in human milk [34], ESI and APCI, both in negative mode, have been used for the determination of APEOs and BPA in milk, meat and honey [41,38,44].

Most studies aimed at detecting and measuring perfluoroalkyl carboxylates and perfluoroalkyl sulfonates use liquid chromatography/tandem mass spectrometry (LC–MS/MS). Gas chromatography/mass spectrometry (GC–MS) is often used for measuring semi-volatile PFASAs and FTOHs. Furthermore, most studies use external calibration, or use only one to two stable isotope-labeled internal standards, to quantify all analytes, though accuracy can be influenced by matrix effects [79].

4. Occurrence of endocrine-disruptor pesticides in food commodities

4.1. Occurrence of industrial chemicals in Crops

The occurrence of industrial endocrine-disrupting compounds in crops is mainly led by pesticides. However, industrial chemical have been found in fruit and vegetables in some studies which will be commented in this section. The origin of the presence of industrial chemicals in crops can be due to different reason, such as the usage of packaging, through contaminated water, or in the case of surfactants, they can be added to pesticide formulations to enhance the stability of pesticide suspensions and emulsions. Surfactants in pesticide formulation increase the leaf retention of spray solutions; enhance herbicide effectiveness and promote the adhesive forces of aqueous droplets on the surface of crop leaves.

Fruits (apple, nectarine, pear, plum, guava, tomato and grape) and vegetables (carrot, cucumber, lettuce, green, pepper, broccoli, celery, spinach, mushroom, and alfalfa sprout) purchased from supermarkets in Taiwan, were analyzed to determine the concentration of

alkylphenolic residues [46]—4-NPs isomers were detected in all the fruit samples, except guava, at concentrations from 3.7 to 16 μg/kg. No alkylphenolic residues were detected in most selected vegetables, except broccoli, and the concentrations of 4-t-OP and 4-NPs were 0.4 and 4.8 μg/kg fresh weight respectively. The varying concentrations of 4-t-OP and 4-NPs reveal that these compounds had found their way into food through miscellaneous routes and at different stages of the food production process. Some may have originated from APEOs, which are used as non-ionic surfactants in disinfectants or as emulsifiers in pesticide formulations. Following their use in agriculture, the degradation products of APEOs could promote the accumulation of 4-t-OP and 4-NPs on the peel of the fruits and vegetables. Another possible source might be from the food-contact plastic materials, which contain tris(nonylphenol) phosphate (TNPP) as antioxidant stabilizers. TNNP is manufactured by the reaction of 4-NPs with phosphorous trichloride. In this way, the degradation products or the impurities of NP residues from TNPP in the plastic may migrate into fruits and vegetables when used in food contact applications.

PCDD/F and PCBs have been analyzed in vegetable oil [47] and concentrations ranged between 0.05 ng/kg and 6.60 ng/kg for PCDD/F and between 0.63 ng/kg and 9446.8 ng/kg for PCBs.

Commercial olive oil samples (extra virgin olive oil, olive oil-composed from refined olive oils and virgin olive oils) from Cosenza (Italy) were analyzed to determine the concentration of phthalates. DEHP was found in olive-pomace oils with a medium concentration of 2.84 mg/kg, this concentration would exceed the SLM established which is 1.5 mg/kg; in extra virgin olive oil and in olive oil, an average concentration of 0.85 mg/kg and 1.45 mg/kg was found, respectively. Lower concentrations of DBP and BBP were found. The concentration of DBP, BBP and DEHP was higher in refined oils and lower in extra virgin oils. Bisphenol A has been found in canned fruit and vegetable samples at levels between 18.4 ng/g and 95 ng/g [48], 29–458 ng/ml [49], 9–48 ng/g [50], 12–24 ng/g [51], 5–35 ng/g [52].

4.2. Occurrence of industrial chemicals in products of animal origin

Animal-origin products considered in this section are meat, fish, milk and milk products, eggs and honey. Human milk, powdered milk, infant milk formula and other baby food products have not been considered in this review.

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and plychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are widespread contaminants with important implications for environmental and human health. Dietary intake, especially the consumption of marine organisms, is considered to be the main route to human exposure from these compounds.

PCBs are mainly analyzed in marine products; however there are some studies of PCBs in other animal-origin products like milk, eggs or meat.

PCBs have been analyzed in farmed tuna from South Australia: concentrations in filets on a fresh weight basis was found at 1281 ng/kg (the sum of 12 PCB congeners) after 496 day of farming—the largest contributor to the sum was PCB 118 [53].

In edible marine crustaceans from the Brittany and Normandy coasts (France) [33], the minimum and the maximum concentrations reported in edible crab were 0.2 ng/kg (PCB 81 congener) and 7621 ng/kg (PCB 153 congener), respectively.

Levels of PCBs in foods from different market in Catalonia (Spain) were studied [24], the concentration expressed as the sum of 11 PCBs congeners were as follow: 11,864 ng/Kg in fish and shellfish, 373.55 ng/Kg in meat, 475,18 ng/Kg in eggs, 674,50 ng/Kg in milk and dairy products and 421.53 ng/Kg in fats and oils.

The concentrations (in μg/kg wet weight) found in salmon and butter purchased from retail shops in different towns over a wide geographical distribution in different European countries [23] were in salmon and butter, respectively, as follow: 40.40 and and 11.48 in Italy, 10.9 and 3.23 in Belgium, 47.80 and 1.86 in Portugal and 36.44 and 4.77 in Spain. Butter was generally of national origin while salmon was generally imported from Norway or Scotland. These concentrations correspond to the sum of 18 PCBs congeners.

In farmed and wild sea bass from Italy [32], the concentration reported as the sum of 59 PCBs was 17.8 and 9.45 ng/g whole weight, respectively.

In beef, chicken, butter and seafood from Egypt [29], the sum of 10 PCBs congeners (expressed in ng/Kg wet weight) were 47 in beef, 184.5 in chicken, 823.5 in butter, 278 in bolti fish, 1019 in mullet, 240 in crab and 198 in bivalves—the main contributor to the sum was the congener 118 in all cases except in chicken samples, for which the main contributor to the sum was congener 189.

PBDEs have been found in edible marine crustaceans from the Brittany and Normandy coasts (France) [33] at concentrations of 0.1 ng/kg in mussels and 8736 ng/kg in sea bass.

PCDD/Fs have been analyzed in farmed tuna from South Australia—concentrations in filets on a fresh weight basis were found at 1.05 ng/kg (the sum of 7 PCDDs and 10 PCDF congeners) after 496 day of farming [53].

PCDD/Fs have been found in edible marine crustaceans from the Brittany and Normandy coasts (France) [33] in concentrations of 0.01 ng/kg in spider crab and 56.03 pg/g in mussels.

In beef, chicken, butter and seafood from Egypt [29], the sum of PCDDs/PCDFs (expressed in ng/Kg wet weight) were 0.6 in beef, 0.5 in chicken, 0.3 in butter, 0.2 in bolti fish, 0.2 in mullet, 0.4 in crab and 1 in bivalves.

Wild caught and farm-raised fish fillets collected in fish markets and large-chain super markets located in Maryland, Washington DC and North Carolina [53] were measured for their polybrominated diphenyl ether (PBDE), polychlorinated biphenyl (PCB), and plychloridebenzo-p-dioxins/dibenzofurans (PCDD/Fs) levels. PCB and PBDE concentrations were the highest in a wild bluefish fillet (800 and 38 μg/kg wet weight, respectively) and lowest in wild Coho salmon fillet (0.35 and 0.04 μg/kg, respectively). Levels for both PCBs and PBDEs in μg/kg wet weight decreased from bluefish with medians of 200 and 6.2 to rockfish, 66 and 4.7, followed by farmed-raised salmon, 9.0 and 1.1 whilst the lowest was in wild salmon, 4.0 and 0.3 μg/kg for PCBs and PBDEs, respectively. PCBs were the sum of 25 congeners.

Phthalate esters are widely used as additives in manufacturing of polyvinyl chloride (PVC) plastics to make them flexible. Extensive use of these chemicals results in their presence in various environmental matrices such as water, soil and food, including milk and other dairy products.

Phthalate has been detected in cow's milk collected at a diary farm in Canada at levels between 0.39 μg/kg (diethyl phthalate DEP) and 282 μg/kg, (di(2-ethylhexyl) phthalate DEHP) [54].

Among the phthalate esters, DEHP is the most commonly used plasticizer worldwide. DEHP is released into the environment through volatilization and leaching from plastics and other sources. DEHP levels in Danish retail whole milk samples were found at up to 0.14 mg/Kg [54].

A survey of Canadian dairy products and margarine has shown that levels of DHPE, butyl phthalate and butyl-benzyl phthalate (BBP) were found at up to 11.9 and 47.8 mg/Kg, respectively [55].

A survey of Japanese retail foods (butter and milk, among other non-animal origin products) [25] revealed the presence of BBP at 0.056 μg/g and DHEP at 2.83 μg/g in butter, and DHEP at the 0.1 μg/g level in milk.

A study for the determination of phthalates in milk containing different packaging and different sterilization systems [40] revealed

the presence of these EDCs in concentrations ranging between 0.97 μg/kg and 85.3 μg/kg; the concentrations were independent of the packaging and the sterilization processes.

Bisphenol A is widely used in the production of numerous resins as well as for the production of polycarbonate plastics and flame retardants [19,56]. Polycarbonate plastics are used in food and drink packaging; resins are used as lacquers to coat metal products such as food cans, bottle tops and milk containers. The migration of BPA from epoxy coated can surfaces, polycarbonate plastics, and PVC products into food has been reported [57,58].

Bisphenol A has been analyzed in 107 honey samples from different countries [44] packaged in glass or plastic containers, and has been detected in 16% of analyzed samples at a concentration between 0.2 μg/kg to 33.3 μg/kg; the findings were not dependent on the kind of container. From these results, there is no reason to speculate that the migration of BPA from food containers has occurred. Alternatively, it can be argued that the contamination present could have occurred during the making, transportation or refining processes of honey.

Bisphenol A has been found in samples of whole milk in concentrations ranging between 0.99 μg/kg and 2,64 μg/kg. The concentrations were independent of the type of packaging and sterilizing process [40].

In China, a study was performed in commercial samples of meat and fish for the determination of nonylphenol, octylphenol and bisphenol A [38]. Nonylphenol was ubiquitous in different types (pork, mutton, chicken, beef, duck) of meat and fish at levels ranging from 0.49 to 55.98 μg/kg. Bisphenol A was found in some of the analyzed samples at levels from 0.33 to 7.08, whilst octylphenol was only found in fish samples.

The major source of NP residues in food packaging comes from oxidation of trisnonylphenyl phosphate (TNPP), used as an antioxidant in polymeric materials [poly-(vinyl chloride) (PVC), polyolephins, and acrylics] and migration from PVC films and jars to milk.

Nonylphenol (NP) was determined in milk at concentrations from 0.4 to 81 μg/kg and was found to be ubiquitous in different types of food at levels between 0.1 and 19 μg/kg [60,61].

The presence of NP and OP has been studied in sea bass [31], the maximum concentration of nonylphenol ranged between 360 and 1554 μg/kg. However, concentrations of octylphenol ranged between 6 and 84 μg/kg.

The presence of hydroxylated-PAHs have been studied in sea bass [31] and concentrations were between 5 μg/kg (1-pyrenol) and 283 μg/kg.

A study of alkylphenol (NP and OP) and octylphenol ethoxylate (OPE) contamination in crustaceans and fish from different areas of the Adriatic Sea (Italy) was performed by Ferrara et al. [63]. NP was often detected at levels as much as 10 times higher than OP and OPE and this reflects its wider use. A strong interspecies variability in NP concentrations was evident. Indeed, it was found at very high levels in anchovies, mackerel and red mullets (988 μg/kg, 954 μg/kg and 723 μg/kg, respectively) but at levels 3–10 times lower in hake, sole and angler fish (31 μg/kg, 47 μg/kg and 69 μg/kg).

A study of alkylphenols, and their ethoxylates, in seafood from the Tyrrhenian Sea [43] revealed that NP is generally detected at higher concentrations. Among the tested species values of total alkylphenols and alkylphenol ethoxylates (44–55 μg/kg and 27–252 μg/kg fw) so far, the maximum concentration was found in shrimps from Fiumicino (1255 μg/kg fw). Conversely, the lowest concentrations were observed in hake and anchovies (34–36 ng/g and 6–370 μg/kg fw). Tuna exhibited very high concentrations of total alkylphenolic compounds (889 μg/kg fw).

Fish and seafood generally contained measurable levels of PFCs. Several studies have shown that fish and seafood accounted for $>50\%$ of PFOS exposure in non-occupationally exposed populations in Canada, Spain, and Poland [80].

5. Concluding remarks.

Analytical methodology reported for determination of industrial chemical in food is more extensive in animal origin food than in crops, as consequence of the physicochemical properties of the studies compounds and the entry routes in the food chain.

The analytical methodology employed in the analysis of industrial chemicals with an endocrine-disruption action in crops and in animal-origin food is based mainly on LC–MS and GC–MS.

With regards to the extraction methods, for crops, the most common is solid-liquid extraction with AcN. However for animal origin food there is more variety in extraction methods, most of them are based in extraction with solvent with especial conditions of temperature and/or pressure (soxhlet and ASE). In general the extractions of food of animal origin are followed by elimination of fat.

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