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Organochlorine Pesticides in Human Serum

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1. Introduction

Organochlorine pesticides are a group of chlorinated compounds that persist in the environment. They were used mainly in agriculture and for pest control in cities for several decades. Their widespread use has resulted in their ubiquitousness in various environments and bioaccumulation throughout the food chain. Although many organochlorine pesticides are banned, their accumulation can have adverse effects on human health and the environment. In this chapter, we emphasize the organochlorine pesticides listed by the Stockholm Convention on persistent organic pollutants: aldrin, chlordanes, dieldrin, endrin, heptachlor, heptachlor epoxide, hexachlorobenzene (HCB), mirex, dichlorodiphenyltrichloroethanes (DDTs), toxaphene, and hexachlorocyclohexanes (HCHs).

As these organochlorine pesticides have similar physicochemical properties, they can be analyzed by using the same analytical procedures, which are composed of extraction, cleanup, and selective and sensitive detection via gas chromatography with an electron capture detector, mass spectrometry, and high resolution mass spectrometry. The application of gas chromatography and high resolution mass spectrometry with an isotope dilution method provides highly accurate and reliable data. We describe a comprehensive extraction and purification method for analysis of target organochlorine pesticides and a measurement method using several equipments later in this chapter.

Many studies have revealed that the general population (including neonates), who lack occupational exposure to organochlorine pesticides, is exposed to background environmental levels of these chemicals. Among the organochlorine pesticides investigated, *p,p'*-DDE, β -HCH, hexachlorobenzene, *p,p'*-DDT, oxychlordane, and *trans*-nonachlor were frequently detected in human serum which are comparatively convenient human biological specimens obtained from all individuals. Epidemiologic research has revealed that these chemicals are associated with several health disorders. Therefore, we conclude this chapter with a summary and critical review on the status of organochlorine pesticides in serum of the general human population.

2. Characteristics of organochlorine pesticides

Organochlorine pesticides have similar chemical structures, showing chlorine-substituted aliphatic or aromatic cyclic rings. Because of the structural similarities, these pesticides share certain physicochemical characteristics such as persistence, toxicity, bioaccumulation, and long-range transport potential. As a result, the Stockholm Convention considered some organochlorine pesticides as environmental hazards and listed them as persistent organic

pollutants. The Convention is global treaty to protect human health and the environment from persistent organic pollutants.

Organochlorine pesticides persist in the environment. Persistency is defined as a half-life greater than two months in water or six months in soil and sediment. These chemicals are difficult to degrade into less hazardous substances in the environment. They are lipophilic compounds that tend to bioaccumulate in fatty tissues through the food chain. Bioaccumulation of organochlorine pesticides is defined as a log K_{OW} value greater than five or bioaccumulation factor in aquatic species greater than 5000. These pesticides are water insoluble and semivolatile, enabling their entry in the atmosphere and transport over long distances globally, mainly by air mass movements. They can reach polar or high mountainous regions and are effectively deposited in cold regions by snow through the phenomenon of cold condensation and global distillation (Wania and Mackay, 1995). Shen and Wania (2005) derived and compiled select physicochemical properties, including vapor pressure, water solubility, Henry's law constant (H), octanol-water partition coefficient (K_{OW}), and octanol-air partition coefficient (K_{OA}) of major organochlorine pesticides (Shen and Wania, 2005). These properties have been used to screen the many chemicals for persistent organic pollutants criteria (Muir and Howard, 2006).

2.1 Dichlorodiphenyltrichloroethane

Dichlorodiphenyltrichloroethane (DDT) is the common name of 1,1,1-trichloro-2,2-di-(4-chlorophenyl)ethane. Technical-grade DDT is a mixture of up to 14 compounds (Fig. 1). The active ingredient is *p,p'*-DDT (65–80%). The other compounds include 15–21% of *o,p'*-DDT, up to 4% of *p,p'*-DDD and 1-(*p*-chlorophenyl)-2,2,2-trichloroethanol, and traces of *o,o'*-DDT and bis(*p*-chlorophenyl)sulfone. Othmar Zeidler, an Austrian scientist, first synthesized DDT in 1874. Paul Hermann Müller, a Swiss chemist who received the Nobel Prize in 1948 by the discovery of its insecticidal properties in 1939; it was widely used as an agricultural insecticide after the war. DDT easily degrades into dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), which are more persistent than the parent compound. The half-life of *p,p'*-DDE in humans has been estimated as more than 7 years

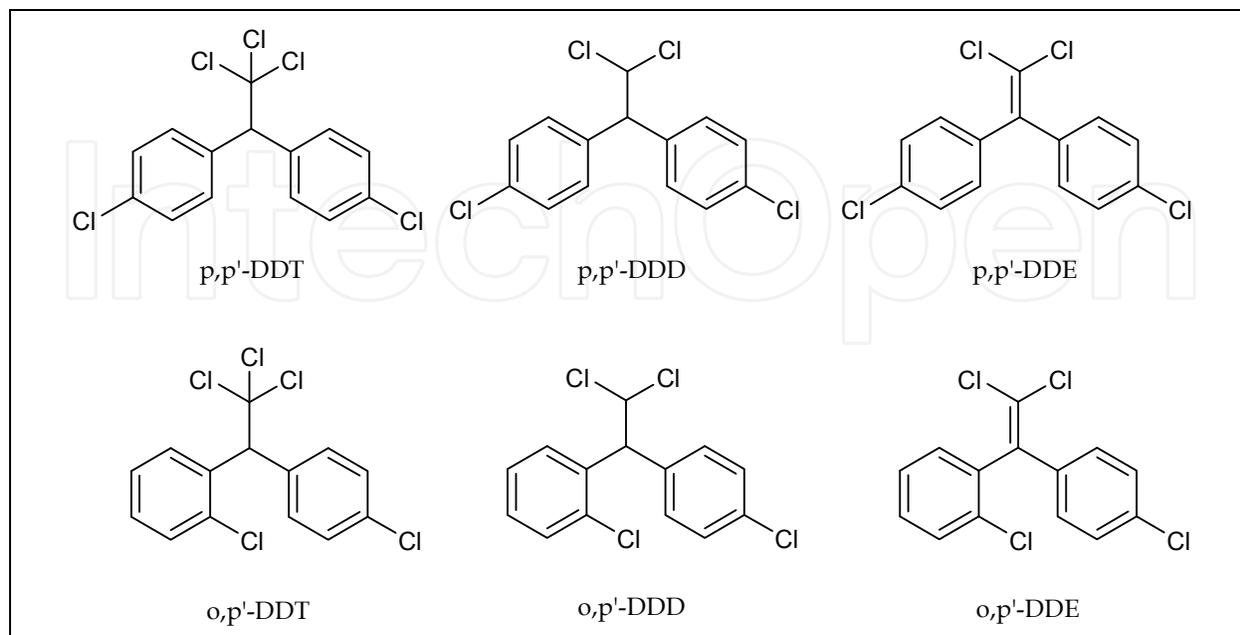


Fig. 1. Structures of dichlorodiphenyltrichloroethane analogues

(Axmon and Rignell-Hydbom, 2006). In the 1970s and 1980s, most countries banned the agricultural use of DDT. It was restricted in the United States in 1972 and finally banned in 1979. Agricultural use continues in some countries, and developing countries use about 4000–5000 tonnes annually for vector control applications.

2.2 Hexachlorocyclohexanes

Hexachlorocyclohexanes (HCHs) are broad-spectrum insecticides used on fruits, vegetables, and forest crops. They are available as two commercial formulations. Technical-grade HCH is a mixture of isomers containing mostly 64% α -HCH, 10% β -HCH, 13% γ -HCH, 9% δ -HCH, and 1% ϵ -HCH (Fig. 2). The other commercial formulation contains more than 99% γ -HCH (lindane). Micheal Faraday discovered HCHs in 1825 and Bender determined their insecticidal properties in 1935. Slade showed that γ -HCH was the only active insecticide of technical-grade HCH in 1942. Technical-grade HCH has been commercially produced since 1947. China and India, the two main users of technical-grade HCH globally, stopped its agricultural use in 1983 and 1990, respectively. The half-life of β -HCH in blood is 7 years, whereas γ -HCH has a half-life of only 20 hours.

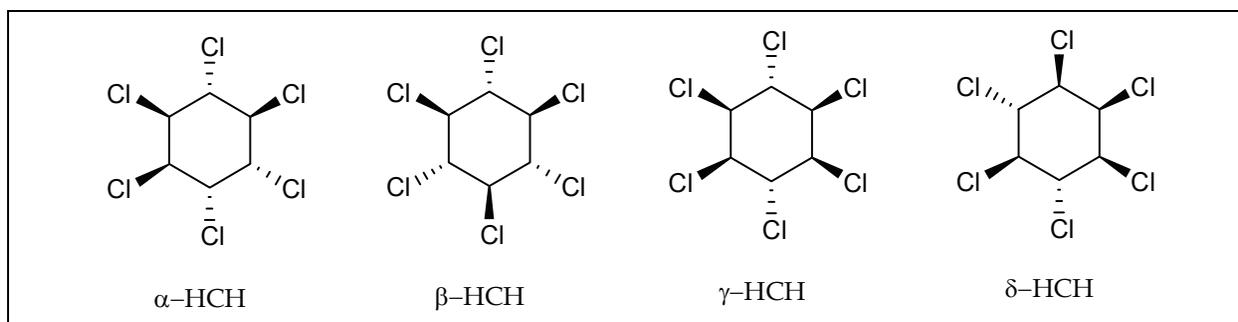


Fig. 2. Structures of hexachlorocyclohexane isomers

2.3 Chlordane

Chlordane was used as a contact insecticide for agricultural crops and lawns, and for termite control in buildings. It has been commercially produced since 1947. Technical-grade chlordane is a mixture of at least 23 compounds and typically consists of 15% *cis*-chlordane, 15% *trans*-chlordane, 9.7% *trans*-nonachlor, 3.9% heptachlor, 3.8% *cis*-nonachlor, other chlorinated hydrocarbons, and by-products (Fig. 3). Nonachlor is an impurity of technical chlordane. Oxychlordane is an oxidized form of chlordane. In the United States, the use of chlordane on food crops was ceased in 1978 and all uses were banned after 1988.

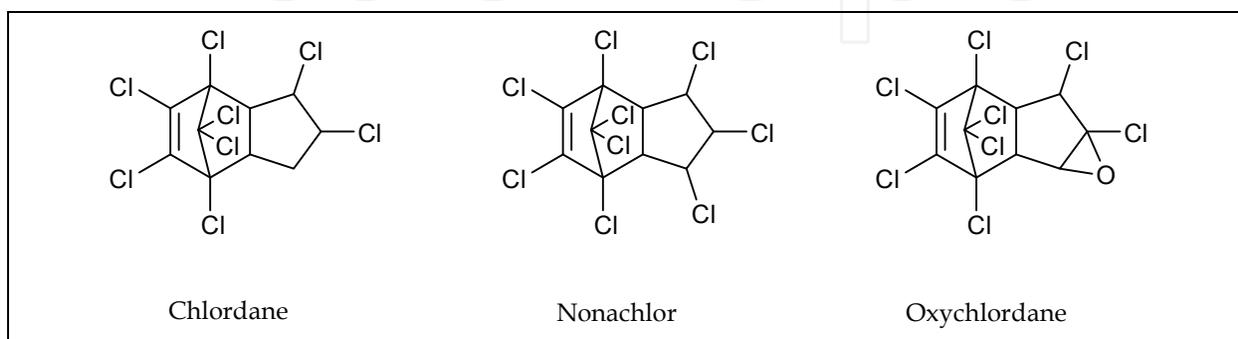


Fig. 3. Structures of chlordane, nonachlor, and oxychlordane

2.4 Heptachlor and heptachlor epoxide

The chemical structure of heptachlor is similar to that of chlordane (Fig. 4). Heptachlor has been used as an insecticide to control fire ants. It is rapidly oxidized by both photochemical and biological processes to heptachlor epoxide, which is an oxidation product of heptachlor. Thus, heptachlor epoxide appears after the use of heptachlor. Heptachlor use was voluntarily discontinued in 1987 in the United States.

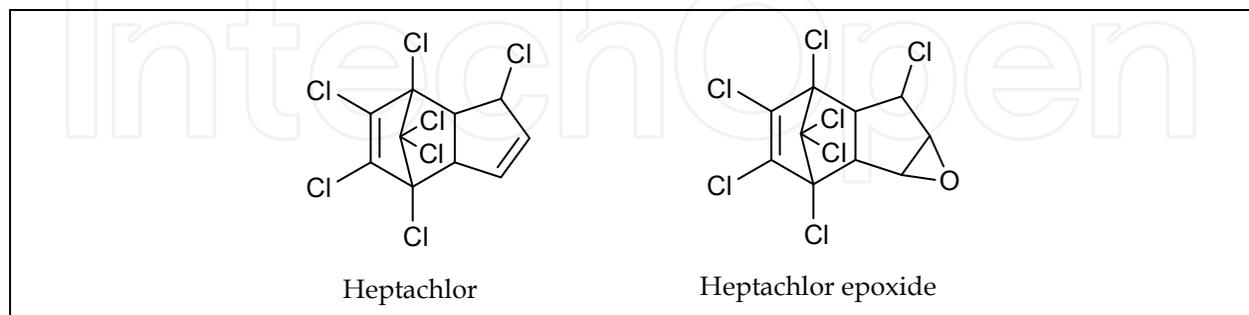


Fig. 4. Structures of heptachlor and heptachlor epoxide

2.5 Aldrin, dieldrin, and endrin

Aldrin, dieldrin, and endrin were used as insecticides from the 1950s to the mid-1970s. They have a similar structure (Fig. 5). Aldrin was first synthesized in 1948 and commercially manufactured in 1950. It is the common name of 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4-endo-5,8-dimethanonaphthalene, and technical-grade aldrin contains 90% of aldrin. Dieldrin, a pesticide product, is the oxygenated metabolite of aldrin. Aldrin easily degrades into dieldrin and is therefore rarely detected in the environment. Endrin was introduced in 1951 and primarily used as a cotton insecticide. It is a stereoisomer of dieldrin and is rapidly metabolized in the environment. Endrin aldehyde and endrin ketone are its degradation products. In most countries, aldrin, dieldrin, and endrin are banned for agricultural use and severely restricted for nonagricultural applications. Agricultural use of these chemicals was banned in 1970 and all uses were banned in 1987 in the United States.

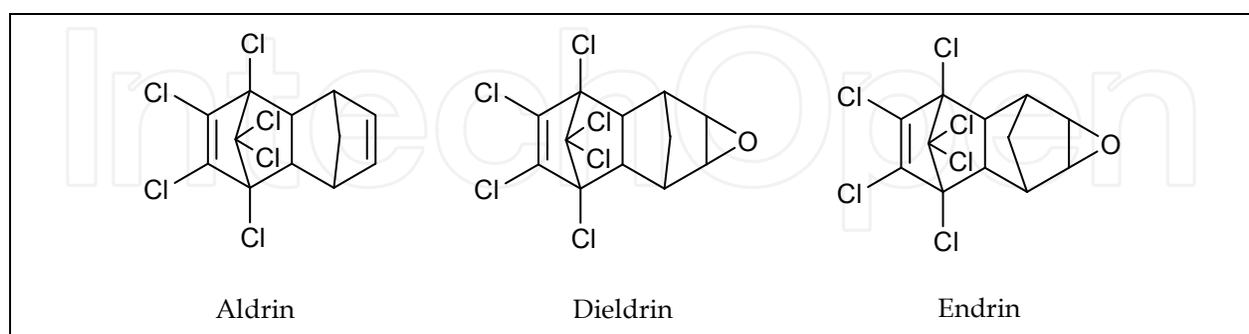


Fig. 5. Structures of aldrin, dieldrin, and endrin

2.6 Mirex

Mirex was synthesized in 1946 and has been used as a pesticide since 1955. It is produced by the dimerization of hexachlorocyclopentadiene in the presence of aluminum chloride (Fig. 6). Mirex was used not only as an insecticide to control fire ants but also as a flame-retardant

in plastic, rubber, paint, paper, and electrical goods. Hooker Chemical developed mirex as a chlorinated flame-retardant under the trade name Dechlorane. Its use as a pesticide was banned in the United States in 1978. Dechlorane was replaced by Dechlorane Plus in 1972.

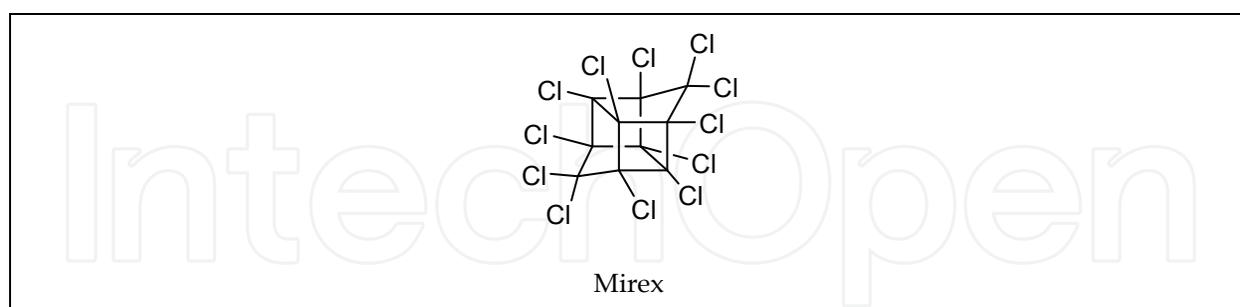


Fig. 6. Structure of mirex

2.7 Hexachlorobenzene

Hexachlorobenzene (HCB) was introduced in 1945 as a fungicide for seed treatment (Fig. 7). It is formed as a by-product or an impurity during chemical and pesticide manufacture, and unintentionally produced by incomplete combustion in municipal solid waste incinerators and industrial processes. Most countries have banned or severely restricted its use as a pesticide. Hexachlorobenzene was banned in the United States in 1984.

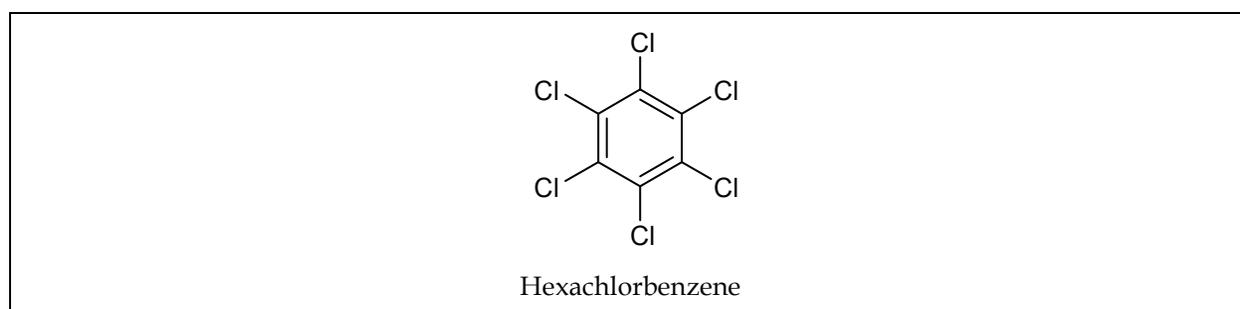


Fig. 7. Structure of hexachlorobenzene

3. Methods for analysis of organochlorine pesticides in human serum

3.1 Sample preparation of serum for analysis

The literature describes several preparation methods to measure organochlorine pesticides in human serum (Barr et al., 2003; Sandau et al., 2003; Focant et al., 2004; Conka et al., 2005; Ramos et al., 2007). The standard method consists of three steps: denaturation, extraction, and cleanup.

Either organic solvents (methanol, propanol, or acetonitrile) or acids (acetic acid, formic acid, and hydrochloric acid) are used for denaturation of the serum protein. As formic acid does not destroy acid-sensitive pesticides such as cyclodienes, it is widely used for denaturation (Sandau et al., 2003). The serum sample can also be denatured with hydrochloric acid and 2-propanol (Hovander et al., 2000).

Serum samples are extracted by using liquid liquid extraction or solid-phase extraction. The conventional method is liquid liquid extraction with an organic solvent such as hexane;

however, this method is laborious and time consuming, and thus unsuitable for large-scale biomonitoring in epidemiologic studies. On the other hand, solid-phase extraction is a simple and efficient method. Commonly, a C₁₈ solid-phase extraction cartridge is used for organochlorine pesticide extraction because the reverse-phase sorbent retains most organic analytes from aquatic matrices. C₁₈ (octadecyl) is bonded to the silica surface to provide nonpolar interactions with the analytes. The Oasis HLB cartridge, a polymeric water-wettable reverse-phase sorbent, can be used for organochlorine pesticide extraction without additional lipid cleanup steps (Sundberg et al., 2006). Each of these sorbents exhibits unique properties of retention and selectivity for organochlorine compounds.

The extract is purified by using several kinds of cleanup methods to remove interferences such as lipids. These methods include conventional glass columns or solid-phase extraction cartridges with neutral silica, florisil, and acid silica. For additional cleanup steps, sulfuric acid treatment, gel permeation chromatography, and active carbon treatment can be added according to interferences. Of the organochlorine pesticides discussed here, heptachlor epoxide, aldrin, dieldrin, and endrin are sensitive to concentrated sulfuric acid. To analyze acid-sensitive pesticides, sulfuric acid treatment and sulfuric acid silica should be avoided during the cleanup steps (Goni et al., 2007). Extraction and cleanup steps with an adequate solid-phase extraction cartridge have advantages such as simplicity, ease of automation, and high throughput.

The typical analytical methods for serum analysis recommended by the Centers for Disease Control and Prevention (CDC) are followed, with minor modifications (Barr et al., 2003; CDC, 2003; Kang et al., 2008). In brief, serum samples (1–2 mL) are spiked with ¹³C-labeled cleanup standards of organochlorine pesticides (ES-5349; Cambridge Isotope Laboratories, USA) and allowed to equilibrate. The samples are denatured and diluted with an equal amount of formic acid and water. The mixtures are remixed to ensure homogeneity and then loaded into preconditioned C₁₈ solid-phase extraction cartridges. Each cartridge is dried and then eluted with 16 mL hexane, followed by concentration of the eluate to less than 6 mL. The eluate is applied to a silica gel/florisil solid-phase extraction cartridge and then eluted with 12 mL hexane followed by 12 mL dichloromethane/hexane (1:1, v/v). The cleaned extracts are concentrated with a gentle stream of nitrogen and transferred to vials. The solvents are evaporated at room temperature with dodecane. Before instrumental analysis, the samples are reconstituted with ¹³C-labeled recovery standards (EC-5350; Cambridge Isotope Laboratories).

3.2 Instrumental analysis

After serum sample preparation, the cleaned extracts are analyzed by gas chromatography with several detectors including an electron capture detector, mass spectrometry, and high resolution mass spectrometry. An electron capture detector is one of the most sensitive detectors to measure halogenated compounds. Gas chromatography with an electron capture detector is employed because of the low cost and ease of operation as well as high sensitivity to organochlorine pesticides. However, this technique cannot differentiate co-eluted compounds. A DB-5 capillary column (30 m height × 0.25 mm inner diameter × 0.25 μm film thickness) is widely used to identify and quantify organochlorine pesticides in human serum. Some pesticides cannot be completely separated in these column conditions. The usual co-eluted organochlorine pesticides are heptachlor epoxide and oxychlordan (Fig. 8).

Gas chromatography with mass spectrometry can resolve the problem of co-elution and avoid misidentification of analytes if operated in the selected ion monitoring mode.

However, the current serum concentrations of some organochlorine pesticides in the general population are too low for quantification by gas chromatography with an electron capture detector or gas chromatography with mass spectrometry. Mass spectrometry allows quantification by isotope dilution. Gas chromatography–high resolution mass spectrometric analysis with an isotope dilution method enables better identification and quantification than the other methods mentioned here. Chemically, the analytes and ^{13}C -labeled analogues behave identically. However, they are distinguishable by their mass differences, allowing complete and automatic recovery correction for each analyte in each sample (Barr et al., 2003). To increase the sensitivity, the selected ion monitoring mode is also used during gas chromatography–high resolution mass spectrometric analysis.

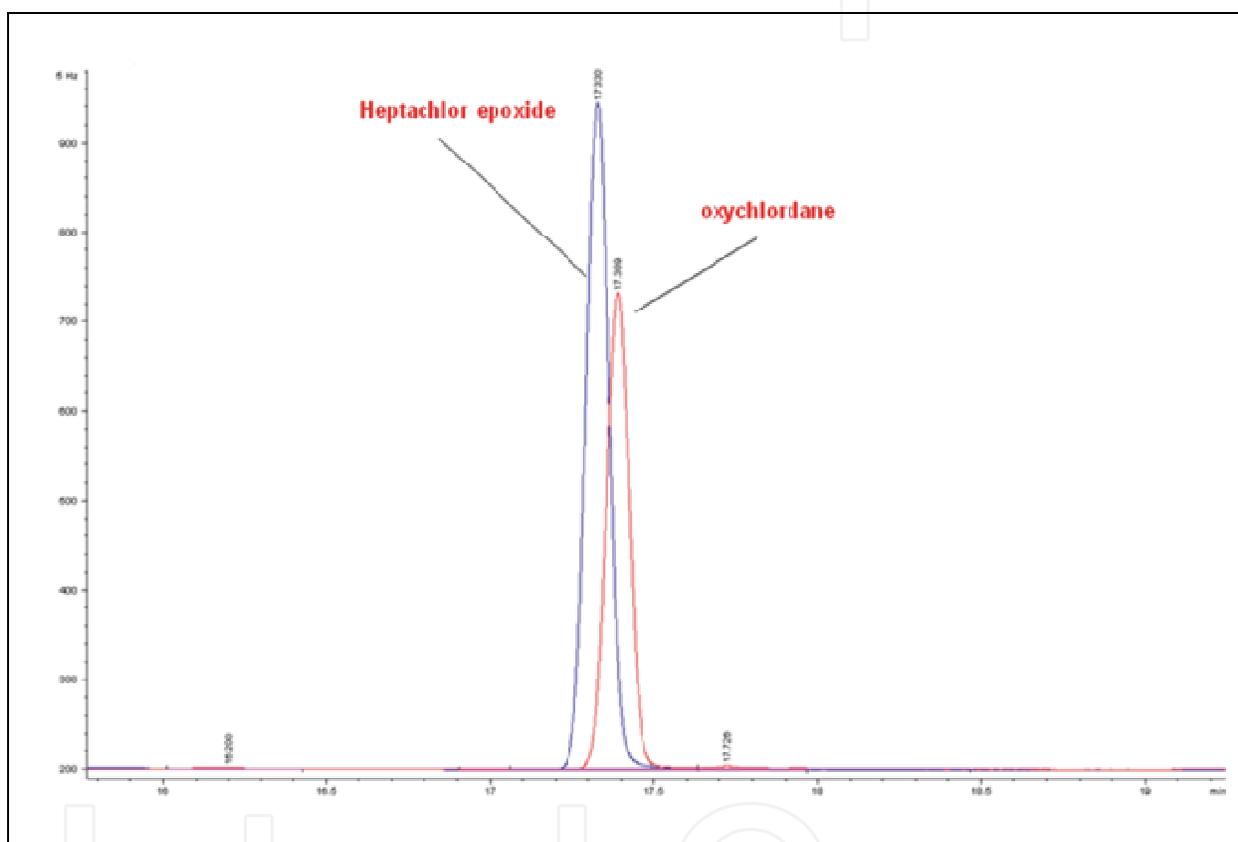


Fig. 8. Overlapping chromatograms of heptachlor epoxide and oxychlorthane by gas chromatography with an electron capture detector

The state-of-the-art analytical method for organochlorine pesticides is isotope dilution gas chromatography–high resolution mass spectrometric quantification. This method is mandatory for PCDD/Fs and coplanar PCB analysis to achieve high sensitivity and precise quantification at low environmental levels. The Centers for Disease Control and Prevention have developed an isotope dilution gas chromatography–high resolution mass spectrometric method for quantification of organochlorine pesticides in human serum samples (CDC, 2003). The isotope-labeled pesticide standards and isotope dilution method support gas chromatography–high resolution mass spectrometric analysis (Kumar et al., 2005). For quantification of 22 persistent organochlorine pesticides, ^{13}C -labeled cleanup internal standards, recovery standards, and calibration standards have been commercially available at Cambridge Isotope Laboratories since 2006 (Kang et al., 2008).

Group	Compound	Monitor	Ion 1	Monitor	Ion 2
Group 1 (15.0-22.3)	HCH	216.9145	[M] ⁺	218.9155	[M+2] ⁺
	¹³ C ₆ -HCH	222.9347	[M] ⁺	224.9117	[M+2] ⁺
	HCB	283.8102	[M+2] ⁺	285.8072	[M+4] ⁺
	¹³ C ₆ -HCB	289.8303	[M+2] ⁺	291.8273	[M+4] ⁺
	Heptachlor	271.8102	[M+2] ⁺	273.8072	[M+4] ⁺
	¹³ C ₁₀ -Heptachlor	276.8269	[M+2] ⁺	278.8240	[M+4] ⁺
	¹³ C ₁₂ -CB-15	234.0406		226.9287	
Group 2 (22.3-23.5)	Aldrin	262.8570	[M+2] ⁺	264.8541	[M+4] ⁺
	¹³ C-Aldrin	269.8805	[M+2] ⁺	271.8775	[M+4] ⁺
	Oxychlorane	386.8053	[M+2] ⁺	388.8024	[M+4] ⁺
	¹³ C ₁₀ -Oxychlorane	396.8388	[M+2] ⁺	398.8358	[M+4] ⁺
	Heptachlor Epoxide	352.8442	[M+2] ⁺	354.8413	[M+4] ⁺
	¹³ C ₁₀ -Heptachlor Epoxide	362.8778	[M+2] ⁺	364.8748	[M+4] ⁺
	¹³ C ₁₂ -CB-70	301.9626		303.9597	
Group 3 (23.5-24.25)	o,p'-DDE	246.0003	[M] ⁺	247.9975	[M+2] ⁺
	¹³ C ₁₂ -o,p'-DDE	258.0406	[M] ⁺	260.0376	[M+2] ⁺
	Chlordane (trans, cis)	372.8260	[M+2] ⁺	374.8231	[M+4] ⁺
	¹³ C ₁₀ -trans-Chlordane	382.8595	[M+2] ⁺	384.8566	[M+4] ⁺
	trans-Nonachlor	406.7870	[M+2] ⁺	408.7841	[M+4] ⁺
	¹³ C ₁₀ -trans-Nonachlor	416.8205	[M+2] ⁺	418.8175	[M+4] ⁺
Group 4 (24.25-25.0)	o,p'-DDD	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	¹³ C ₁₂ -o,p'-DDD	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	p,p'-DDE	246.0003	[M] ⁺	247.9975	[M+2] ⁺
	¹³ C ₁₂ -p,p'-DDE	258.0406	[M] ⁺	260.0376	[M+2] ⁺
	Dieldrin	262.8570	[M+2] ⁺	264.8541	[M+4] ⁺
	¹³ C ₁₂ -Dieldrin	269.8805	[M+2] ⁺	271.8775	[M+4] ⁺
Group 5 (25.0-26.0)	Endrin	262.8570	[M+2] ⁺	264.8541	[M+4] ⁺
	¹³ C ₁₂ -Endrin	269.8805	[M+2] ⁺	271.8775	[M+4] ⁺
	p,p'-DDD	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	¹³ C ₁₂ -p,p'-DDD	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	o,p'-DDT	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	¹³ C ₁₂ -o,p'-DDT	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	cis-Nonachlor	406.7870	[M+2] ⁺	408.7841	[M+4] ⁺
	¹³ C ₁₀ -cis -Nonachlor	416.8205	[M+2] ⁺	418.8175	[M+4] ⁺
Group 6 (26.0-33.0)	p,p'-DDT	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	¹³ C ₁₂ -p,p'-DDT	235.0081	[M] ⁺	237.0053	[M+2] ⁺
	Mirex	271.8102	[M] ⁺	273.8072	[M+2] ⁺
	¹³ C ₁₀ -Mirex	276.8269	[M] ⁺	278.8240	[M+2] ⁺

Table 1. Accurate mass for selected monitoring ions, grouping retention times, and fragments

The conventional gas chromatography–high resolution mass spectrometric conditions are as follows. The separation of organochlorine pesticides is carried out by using a DB-5MS capillary column (60 m height × 0.25 mm inner diameter × 0.25 μm film thickness). A splitless injection (1 μL) is used with an injector temperature of 260 °C. The oven is programmed from 100 °C (5 min) to 280 °C with a ramp rate of 10 °C/min and then held at 280 °C for 10 min. The transfer line and source temperatures are 280 °C in the electron impact mode. The resolution is maintained over 10,000 (10% valley definition). The retention time is divided to collect data on 22 organochlorine pesticides in six groups. The accurate mass of the selected monitoring ions, grouping retention times, and fragments are shown in Table 1. The typical chromatograms of HCHs and DDEs in human serum are presented in Fig. 9.

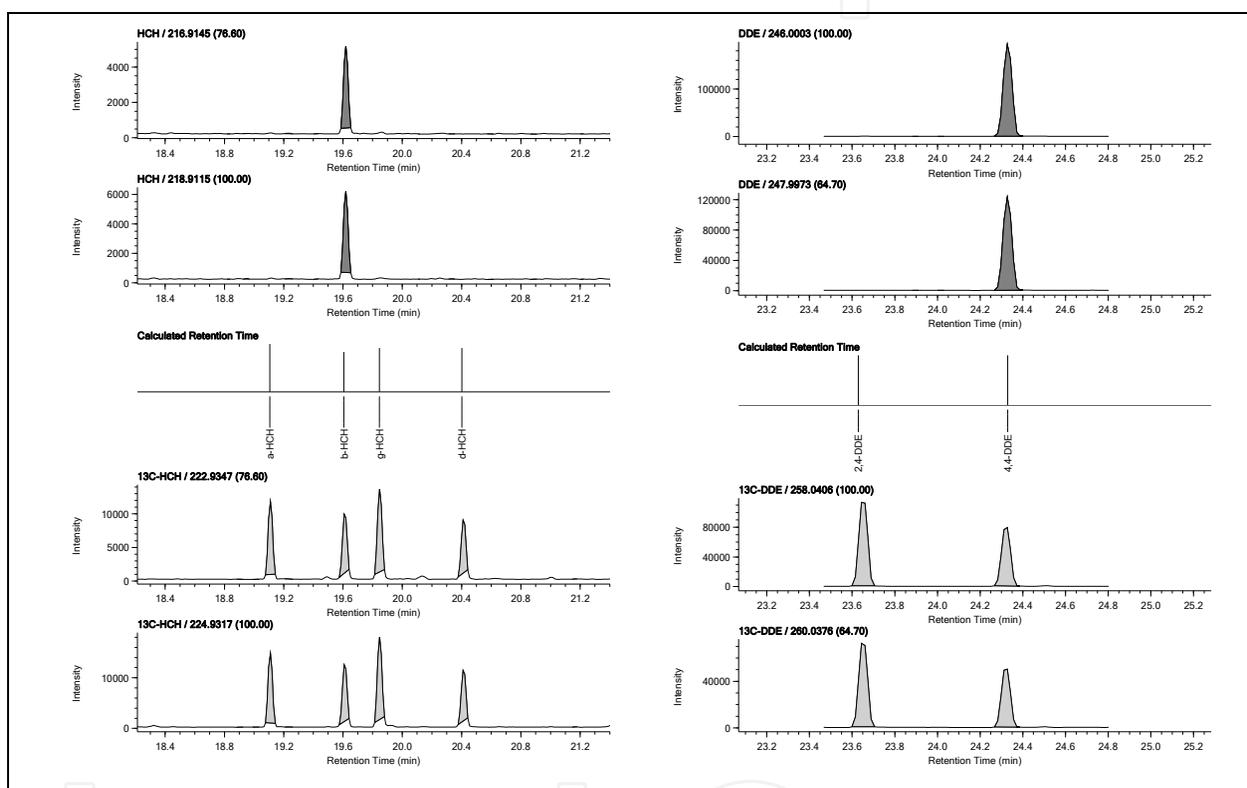


Fig. 9. Gas chromatography–high resolution mass spectrometric chromatograms of hexachlorocyclohexanes and dichlorodiphenyldichloroethylenes in human serum

4. Organochlorine pesticides in human serum

4.1 Organochlorine pesticides in the general population

Everybody has organochlorine pesticides in their body. Irrespective of age, gender, socioeconomic status, and country, these pesticides and their metabolites are detectable in blood or tissue, although the exposure levels differ according to various factors. Reported data on the levels of organochlorine pesticides in human serum are summarized in Table 2. It is difficult to compare these results directly because of the different periods of survey, ranging from 1959 to 2006. However, the serum levels of these chemicals in the general population have been decreasing over time, after the periods of their peak production and use. The most abundant residue in the general population is *p,p'*-DDE (Table 2); its median

serum concentration is much higher in the Chinese general population than in the United States, European, and other Asian populations in the same sampling year. The serum concentrations of these pesticides show regional differences because of regional variations in their use and different times of discontinuation.

Human exposure to organochlorine pesticides is primarily through the diet. The dietary main source is fatty food, such as meat, fish, poultry, and dairy products. Continuous exposure primarily occurs through the food supply, even if the produced foods within a country do not contain detectable residues, because food imported from other countries that still use organochlorine pesticides may be a source of human exposure. Exposure via inhalation of the ambient air is thought to be insignificant compared with dietary intake. Prenatal exposure to organochlorine pesticides is possible via the placenta. Breast feeding is the major source of infant exposure.

4.2 Levels of organochlorine pesticides in the North American population

The National Health and Nutrition Examination Survey (NHANES), conducted by the CDC's National Center for Health Statistics, is a program of studies designed to assess the health and nutritional status of adults and children in the United States. Of the part of the NHANES, organochlorine pesticides were measured in the 1999–2000, 2001–2002, and 2003–2004 surveys in a subsample of participants aged 20 years and older, who were selected as the representative sample of the United States population. The abundant organochlorine pesticides in serum were *p,p'*-DDE, *trans*-nonachlor, oxychlordane, HCB, and β -HCH. In the NHANES 1999–2000 and 2001–2002, the median serum HCB concentrations were below the detection limit, and in the NHANES 2003–2004 survey, the median serum concentration was 14.9 ng/g lipid. The median concentration of *p,p'*-DDE ranged from 203 to 250 ng/g lipid. The serum concentration of this compound was higher in Mexican-Americans than in non-Hispanic blacks and whites. The median serum concentrations of *trans*-nonachlor ranged from 17.8 to 14.8 ng/g lipid between 1999 and 2004. The median concentrations of oxychlordane in 2001–2002 and 2003–2004 were 11.1 and 12.3 ng/g lipid, respectively. The median concentrations of *p,p'*-DDT and *o,p'*-DDT were below the detection limit in all these surveys. The median concentrations of aldrin, dieldrin, endrin, β -HCH, γ -HCH, heptachlor epoxide, and mirex were also below the limit of detection. The serum concentrations of HCB, β -HCH, *p,p'*-DDE, oxychlordane, and *trans*-nonachlor increased with increasing age and were significantly higher in the 60 years and older age group (CDC, 2005; Patterson et al., 2009).

Because the general population was exposed to these pesticides by eating contaminated fatty food such as fish, an Inuit population in Greenland showed high levels of exposure (Rusiecki et al., 2008). Serum samples were collected from 70 Greenlandic Inuit (61 men, 9 women) under the Arctic Monitoring and Assessment Program (AMAP) in 1997–1998. Their age of the subject ranged from 19 to 67 years. The median concentrations of *p,p'*-DDT, *p,p'*-DDE, β -HCH, HCB, *trans*-chlordane, oxychlordane, and mirex were 25.1, 1268, 40.0, 239, 883, 249, and 38.9 ng/g lipid, respectively. Compared with the other populations and three National Health and Nutrition Examination Survey results, the serum organochlorine pesticide levels in Greenland Inuit are relatively high and the *p,p'*-DDE and β -HCH levels rank among the highest worldwide.

The National Health and Nutrition Examination Survey biomonitoring study show the current levels of organochlorine pesticides in the general population of the United States, while a longitudinal cohort study reveal the past exposure levels. The Child Health and Development Studies (CHDS) established a longitudinal cohort of 20,754 pregnant women enrolled in the San Francisco Bay Area Kaiser Foundation Health plan between 1959 and 1967 (Bhatia et al., 2005). During this period, DDT was produced and used in the United States (banned since 1972). Of the cohort, 283 maternal serum samples were randomly selected for DDT and DDE analysis. The median serum concentrations of *p,p'*-DDE and *p,p'*-DDT in maternal serum were 5200 and 1400 ng/g lipid, respectively. James et al. (2002) measured the serum concentrations of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDT, *o,p'*-DDE, and heptachlor epoxide in pregnant women participating in the Child Health and Development Study (CHDS), conducted during 1963–1967, which was the time of peak usage and production of these organochlorine pesticides (James et al., 2002). The median concentrations of *p,p'*-DDE and *p,p'*-DDT were 5878 and 1611 ng/g lipid, respectively. The serum from the women of this cohort showed higher organochlorine pesticide concentrations than those of the recent general population samples. The serum concentration of *p,p'*-DDE and *p,p'*-DDT in the United States dramatically decreased over the period between 1950s and 2000s. The concentrations of *p,p'*-DDE were comparable in the Child Health and Development Study and in Chinese women from Shanghai in 1996–1998, whereas the concentration of *p,p'*-DDT was much higher than that in the Chinese women (Lee et al., 2007b).

4.3 Levels of organochlorine pesticides in the general Asian population

4.3.1 China

Currently, the general Chinese population shows one of the highest organochlorine pesticide residues in their body, indicating recent heavy use of these pesticides. The serum samples of 250 Chinese women from Shanghai were collected in 1996–1998 as part of a population-based case-control study on breast cancer (Lee et al., 2007b). The median serum concentrations of β -HCH, *p,p'*-DDT, *p,p'*-DDE, and HCB, measured by gas chromatography with an electron capture detector, were 5065, 309, 7635, and 62.7 ng/g lipid, respectively. The serum concentrations of β -HCH and *p,p'*-DDE among these women were much higher than those observed in other general populations in the same year. To assess the exposure to persistent organic pollutants in south China, serum samples were collected from the inhabitants of Guiyu and Haojiang, Guangdong Province, in 2005 and analyzed for 12 organochlorine pesticides by using gas chromatography and mass spectrometry in electron impact and selected ion monitoring modes (Bi et al., 2007). The *p,p'*-DDE concentrations ranged from 81 to 1500 ng/g lipid (median concentration of 540 ng/g lipid) in Guiyu and from 320 to 3900 ng/g lipid (median concentration of 1800 ng/g lipid) in Haojiang. The median HCH and DDT concentrations in Haojiang were three times higher than those in Guiyu although these cities are only about 50 km apart; the main industry of Guiyu is e-waste recycling and that of Haojiang is fishing. Guiyu showed one of the highest serum PBDE concentrations in the world. The general Chinese population has the highest concentrations of *p,p'*-DDE, *p,p'*-DDT, and β -HCH in their serum, suggesting that this population was heavily exposed to these pesticides, despite the fact that DDTs and HCHs have been banned in China.

4.3.2 Japan

The background serum levels of organochlorine compounds were evaluated by determination of 8 PCDDs, 10 PCDDFs, 40 PCBs, and 13 organochlorine pesticides in Japanese women of reproductive age (Tsukino et al., 2006). The serum samples were obtained from 80 Japanese women aged 26–43 years and then analyzed by isotope dilution gas chromatography and high resolution mass spectrometry at the CDC in the United States. The frequently detected organochlorine pesticides were *p,p'*-DDE (median concentration of 221 ng/g lipid) followed by β -HCH, *trans*-nonachlor, and oxychlordane. The median concentrations of HCB, γ -HCH, heptachlor epoxide, dieldrin, *o,p'*-DDT, *p,p'*-DDT, and mirex were below the limit of detection. The results of this study firstly revealed the median serum concentration of *p,p'*-DDE in the general Japanese population. This concentration in serum was significantly and positively associated with fish intake. The geographic variation of serum organochlorine pesticide concentrations in Japan was studied by using serum samples obtained from the general population living in three locations (Miyako, Saku, and Nagono) in 1999 (Minh et al., 2006). The plasma samples were prepared by liquid liquid extraction, lipids were removed by a gel permeation chromatography and florisil column, and organochlorine pesticides were measured by gas chromatography with an electron capture detector. There were no significant regional differences in the concentrations of HCHs, DDTs, and HCB. On the other hand, chlordane compounds showed apparent geographic differences. Miyako had higher serum concentrations of chlordanes, suggesting historic use of chlordane pesticides for termite control.

Fukata et al. (2005) assessed fetal exposure to chlorine contaminants. In their study, 32 maternal serum and umbilical cord serum samples were collected from pregnant women who lived in Chiba and Yamanashi, near Tokyo, Japan in 2002–2003 (Fukata et al., 2005). Nineteen organochlorine pesticides were measured by using gas chromatography and mass spectrometry after liquid liquid extraction and a florisil cartridge cleanup. The highest concentrations found in maternal serum were *p,p'*-DDE (median concentration of 93 ng/g lipid), which was the lowest level of this compound among other studies on the general Japan population. The median concentrations of HCB, HCHs, *p,p'*-DDT, oxychlordane, and *trans*-nonachlor were 16, 26, 2.4, 1.2, and 7.0 ng/g lipid, respectively, with a detection rate over 80%. The median concentrations of *p,p'*-DDE and HCB in maternal serum were higher than those in cord serum. This study found a strong correlation between maternal serum and cord serum for some organochlorine pesticides such as HCB, HCHs, and heptachlor epoxide, which showed a correlation coefficient over 0.72; in particular, maternal serum showed higher organochlorine pesticide concentrations than cord serum.

Masuda et al. (2005) collected 152 blood samples from residents aged 20–60 years in Fukuoka in 1999 (Masuda et al., 2005). The serum samples were extracted with acetone/hexane and the lipid was removed by gel permeation chromatography. The samples were analyzed for HCB, *p,p'*-DDE, *p,p'*-DDT, *p,p'*-DDD, β -HCH, dieldrin, *trans*-nonachlor, *cis*-nonachlor, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, and oxychlordane by using gas chromatography–high resolution mass spectrometry. ^{13}C -labeled internal standards for organochlorine pesticides were not used in this study. All the pesticides analyzed were detected in serum samples. The median serum concentrations of HCB, β -HCH, dieldrin, *p,p'*-DDE, *p,p'*-DDT, *trans*-nonachlor, and oxychlordane were over 10 ng/g lipid, whereas those of heptachlor epoxide, *p,p'*-DDD, *trans*-chlordane, *cis*-chlordane,

and *cis*-nonachlor were lower than 10 ng/g lipid. The most abundant compound was *p,p'*-DDE (median concentration of 312 ng/g lipid) followed by β -HCH (median concentration of 280 ng/g lipid), which was the highest levels in Table 2, except for the Chinese samples from Shanghai.

Takasuga et al. (2004a) studied biological elimination of persistent organic pollutants in humans via intake of fermented brown rice with *Aspergillus oryzae* (Takasuga et al., 2004a). After two years of consumption, those who ate the fermented brown rice had greater elimination of PCDD/Fs than those who did not, although the former group showed slightly higher serum PBDE concentrations (Takasuga et al., 2004b). In 18 serum samples from nine couples aged 37–48 years in Japan, selected organochlorine pesticides were identified by gas chromatography–high resolution mass spectrometry to assess the impact of intake of fermented brown rice with *Aspergillus oryzae* on biological elimination of organochlorine pesticides (Takasuga et al., 2006). This paper reported total DDT, total HCH, total chlordane, and HCB concentrations of 230, 53, 36.5, and 13.5 ng/g lipid, respectively. Those who ate the fermented brown rice (7.5–10.5 g after their meal) for two years did not show reduced serum concentrations of DDTs, HCHs, and chlordanes.

4.3.3 Korea

Kang et al. (2008) reported the first investigation on the levels of organochlorine pesticides in human serum samples from urban areas in Korea (Kang et al., 2008). The serum samples were obtained from 40 subjects who participated in the Health Assessment Study of Seoul Citizens related to municipal solid waste incinerators and lived in three areas (Kangnam, Nowon, and Yangchun) in Seoul. The participants consisted of 20 males and 20 females (age range 27–58 years, mean age=45). The serum samples were extracted on C₁₈ solid-phase extraction cartridges and then applied to silica gel/florisil solid-phase extraction cartridges for cleanup. The concentrations of organochlorine pesticides and PCBs were measured by isotope dilution gas chromatography–high resolution mass spectrometry, which gave accurate and precise data for investigations of trend and international comparisons. Among the 22 investigated organochlorine pesticides, *p,p'*-DDE, β -HCH, *p,p'*-DDT, HCB, and *trans*-nonachlor were frequently detected in all samples. The most abundant pesticide was *p,p'*-DDE, having a median concentration of 224 ng/g lipid. The median serum concentrations of β -HCH, *p,p'*-DDT, and HCB were higher than 10 ng/g lipid, whereas *trans*-nonachlor, heptachlor epoxide, oxychlordane, and *p,p'*-DDD had median concentrations below 10 ng/g lipid. The correlation coefficients between PCBs and organochlorine pesticides ranged from 0.365 to 0.906, with the highest correlation found between PCB153 and *trans*-nonachlor ($r = 0.906$). The organochlorine pesticides were also positively correlated to each other. Strong correlations between serum concentrations of organochlorine pollutants suggest that humans are exposed to PCBs and organochlorine pesticides via similar routes, because they were both widely used in the same period. In studies conducted within the Chinese population, extremely high concentrations of organochlorine pesticides contrasted with very low concentrations of PCBs (Lee et al., 2007b). A community-based health survey was performed in 2006 in Uljin (Son et al., 2010). Uljin is a geographically small county located on the east coast of Korea. Among 1007 participants, 40 subjects with diabetes and 40 controls matched for sex and age were selected. The most abundant organochlorine pesticide was *p,p'*-DDE,

followed by β -HCH, *p,p'*-DDT, *trans*-nonachlor, HCB, oxychlorane, and heptachlor epoxide among the 22 organochlorine pesticides analyzed. The total organochlorine pesticide concentrations ranged from 38.8 to 4598 ng/g lipid, with the mean and median concentrations of total organochlorine pesticides being 638 and 483 ng/g lipid, respectively. In addition, when the concentrations of organochlorine pesticides were compared between Seoul and Uljin, regional differences were found.

4.3.4 New Zealand

The serum samples were obtained from the New Zealand population aged 15 years and older during 1996–1997 (Bates et al., 2004). This is the first persistent organochlorine biomonitoring study in the adult population of an entire country, and the 60 serum samples were pooled according to stratification criteria by using 1834 individual serum samples. The aim of the study was to estimate the baseline concentration of organochlorine pesticides in serum among the general New Zealand population. Organochlorine pesticides were extracted by using a C₁₈ solid-phase extraction cartridge and purified by a florisil cartridge. Target analytes were quantified by isotope dilution gas chromatography–high resolution mass spectrometry operating in the selected ion monitoring mode. The frequently detected pesticides were *p,p'*-DDE, β -HCH, dieldrin, *p,p'*-DDT, HCB, and *trans*-nonachlor, but aldrin, endrin, heptachlor epoxide, oxychlorane, and mirex were not detected in the pooled serum. The median concentrations of *p,p'*-DDE and β -HCH were 919 and 10.7 ng/g lipid, respectively. The *p,p'*-DDE concentration showed an increasing trend from the north to the south regions, reflecting historical patterns of DDT use. In this study, dieldrin was frequently detected, with a median concentration of 11.5 ng/g lipid. The use of DDT and dieldrin in agriculture was banned in the 1970s. The serum concentrations of pesticides increased with age. The concentrations of *p,p'*-DDE, β -HCH, and dieldrin were significantly higher in the group aged over 50 years than in the younger age group. The median *p,p'*-DDE concentration of these subjects was lower than that of subjects from China (Bi et al., 2007), Romania (Dirtu et al., 2006), and Slovakia (Petrik et al., 2006). However, the median concentration of this compound in the New Zealand population was higher than that in subjects from Korea (Kang et al., 2008), Japan (Tsukino et al., 2006), the United Kingdom (Thomas et al., 2006), and the United States (CDC, 2005).

4.4 Levels in the European population

4.4.1 Sweden

In a population-based case–control study of organochlorines and endometrial cancer risk, the serum concentrations of five organochlorine pesticides in elderly women in Sweden were reported to evaluate associations between serum concentrations of organochlorines and lifestyle or medical characteristics of Swedish women (Glynn et al., 2003). The 205 women aged 50–74 years old were participated as controls from 12 Swedish counties on the coasts of the Gulf of Bothnia, the Baltic Sea, and the largest Swedish lakes in 1996–1997. The frequently detected organochlorine pesticides, *p,p'*-DDE, HCB, β -HCH, and *trans*-nonachlor, and oxychlorane, were analyzed on gas chromatography with an electron capture detector. The most abundant pesticide was *p,p'*-DDE with the median concentration of 497 ng/g lipid. The other pesticides had median concentrations below 10 ng/g lipids. Correlation analysis

showed that the concentrations of chlorinated pesticides and their metabolites were positively correlated with each other ($r = 0.48-0.89$). High correlation was found between oxychlordane and *trans*-nonachlor. Age was a significant determinant of serum concentration of chlorinated pesticides in this study. Glynn et al. (2007) also reported another serum concentration of the general Swedish population (Glynn et al., 2007). The serum of 323 pregnant primiparous women living in Uppsala county (age 18-41 years) sampled in 1996-1999. Organochlorine pesticides and their metabolites were analysed by gas chromatography with an electron capture detector. Concentrations of α -HCH, γ -HCH, oxychlordane, *o,p'*-DDT, *o,p'*-DDD, *p,p'*-DDT, and *p,p'*-DDD were below the limit of quantification, indicating no recent exposure to technical mixtures of these pesticides. Median concentrations of β -HCH, HCB, *trans*-nonachlor and *p,p'*-DDE were 9, 23, 5, and 88 ng/g lipid, respectively. Compared to previous study, median concentration of *p,p'*-DDE was extremely different. It could be explained that different consumption patterns of fish. More than half of the women reported they did not eat fish from the Baltic Sea in this study. Regression analysis showed that women born in the Nordic region had higher concentrations of β -HCH and *p,p'*-DDE compared to the non-Nordic region. Concentrations of β -HCH, HCB, *trans*-nonachlor and *p,p'*-DDE were also increased with increasing age and positively associated with consumption of fatty fish during adolescence.

4.4.2 United Kingdom

The serum was collected from 154 volunteers aged 22 to 80 from 13 UK cities and towns in 2003 (Thomas et al., 2006). Because the serum extract was cleaned using concentrated sulfuric acid, 12 organochlorine pesticides were analyzed by gas chromatography and mass spectrometry (*trans*-chlordane and *cis*-chlordane, HCB, six DDT analogues, α -HCH, β -HCH, and γ -HCH). The median concentrations of HCB, β -HCH, and *p,p'*-DDE were 11, 12, and 100 ng/g lipid, respectively. The concentration of *p,p'*-DDE showed relatively wide range from 15 to 2600 ng/g lipid. The median concentrations of *p,p'*-DDE in UK was lower than those found in Belgium (Covaci et al., 2002; Koppen et al., 2002), the United States (CDC, 2005), and similar to Sweden (Glynn et al., 2003). β -HCH and *p,p'*-DDE concentration also showed that significant and positive correlation with age.

4.4.3 Spain

The 682 serum samples of the Spanish population from the Canary Islands was collected in 1998 (Zumbado et al., 2005). The study subjects aged between 6 and 75 years and lived in the Canary Islands, where are extensive farming areas and compose of seven islands. Investigated organochlorine pesticides were determined by gas chromatography with an electron capture detector. Of the DDT analogues, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDE, *o,p'*-DDD, and *o,p'*-DDT were detected in the serum samples. Even technical DDT prohibited nowadays, *o,p'*-DDT and *p,p'*-DDT were frequently present in 40% of the samples. The median concentrations of *p,p'*-DDE was 118 ng/g lipid, while the total DDT serum concentration was 380 ng/g lipid. Of the seven islands (Gran Canaria, Lanzarote, Fuerteventura, Tenerife, La Palma, La Gomera, and El Hierro), median serum concentrations of *o,p'*-DDT and *p,p'*-DDT from Gran Canaria showed 250 and 233 ng/g lipid, respectively, while the median concentrations of those compounds from the other

regions were not detected over limit of the detection. However, serum concentration of *p,p'*-DDE showed between 94 and 140 ng/g lipid, which is consistent according to the islands. Luzardo et al. (2006) reported the serum concentrations of lindane and cyclodienes (aldrin, dieldrin and endrin) in the young population of the Canary Islands (Luzardo et al., 2006). Lindane, aldrin, and endrin were detected with the detection frequency of over 50% of the samples and the median concentrations were 69.9, 54.5, and 34.9 ng/g lipid, respectively. Endrin was the most frequently detected cyclodienes (72%), and dieldrin has 27% of detection rate in this population. Even this study reported for the first time the presence of cyclodiene and lindane in a Spanish population, lindane, aldrin, and endrin were not commonly detected pesticides in other previous studies. The serum of 72 women living in El Ejido and Granada in Spain were collected and the subjects aged from 18 to 35 years (Jimenez Torres et al., 2006). At the same time their adipose tissue and umbilical cord blood were also collected during giving birth by caesarean section to establish a correlation in organochlorine compounds between these biological compartments. The serum mean concentrations of lindane and HCB were 1.3 and 20.1 ng/mL serum. The serum mean concentrations of *p,p'*-DDE, *p,p'*-DDD, and *p,p'*-DDT were 31.9, 44.5, and 10.5 ng/ml serum, respectively. The most abundant contaminated pesticides were endosulfan II, followed by *p,p'*-DDE. The concentration of *p,p'*-DDE in between maternal serum and umbilical cord serum was significantly different ($p < 0.001$). Of the EPIC (European Prospective Investigation into Cancer and Nutrition) Spain cohort, serum samples consisted of 953 subjects aged 35-64 years old from five Spanish regions during 1992-1996 (Jakszyn et al., 2009). The serum were analyzed using gas chromatography with an electron capture detector to quantify β -HCH, HCB, *p,p'*-DDE, and *p,p'*-DDT. The median concentrations of β -HCH, HCB, *p,p'*-DDE were 221, 462, and 857 ng/g lipid, respectively. Increasing level of *p,p'*-DDE and β -HCH to southern region in the Mediterranean coast at the south of Spain, while level of HCB increasing to the north region. After adjusting by age, gender, region, body mass index (BMI), and sampling year, serum concentrations of organochlorine pesticides were not associated with any dietary consumption patterns.

4.4.4 Denmark

The Faroese birth cohort consisted of 1022 children in the Faroese islands during 1986-1987, which have been carried to characterize the adverse effects of seafood contamination and assess the prenatal exposure of the contaminants (Barr et al., 2006). Serum samples were analyzed for PCBs and *p,p'*-DDE at delivering time and age 14 years. The median serum concentration of *p,p'*-DDE for the cord blood was 71 ng/g lipid, and for 14 years old children was 467 ng/g lipid. The concentrations of *p,p'*-DDE in umbilical cord and children at age 14 showed significantly different concentrations, which were increased by age. The correlation analysis by the Pearson correlation coefficients showed that the *p,p'*-DDE was highly correlated with highly chlorinated PCB congener, PCB 180.

4.4.5 Belgium

Of the Flemish Environment and Health Study (FLEHS), serum samples were obtained from 200 Flemish women living in Antwerp (urban area) and Peer (rural area) in 1999 (Koppen et al., 2002). The 47 pooled serum samples were made by mixing of individual serum sample.

The pooling categorized by ranking the women in the order of four criteria; decreasing daily intake of meat and fish, decreasing daily intake of eggs and milk, increasing total number of weeks of lactation and increasing BMI. The pooled serum samples were spiked with internal standard and added with formic acid. The mixtures were subjected to extraction using solid-phase extraction cartridge, followed by cleanup on acidified silica gel cartridge. HCB, *p,p'*-DDT, *p,p'*-DDE, and γ -HCH were measured using gas chromatography and mass spectrometry. The median concentrations of HCB, *p,p'*-DDT, *p,p'*-DDE, and γ -HCH were 109.9, 2.6, 871.3 and 5.7 ng/g lipid, respectively. Concentrations of *p,p'*-DDE, and γ -HCH were higher in rural region (Peer). As the use of DDT has been banned more than 30 years ago, concentrations of *p,p'*-DDT were significantly higher in Peer. HCB was significantly higher in urban area (Antwerp). This study reported γ -HCH concentration in serum samples.

5. Effects of organochlorine pesticides on human health

Most organochlorine pesticides are known to be endocrine disruptors, neurotoxicants, and carcinogens. Their presence in the general population at the current background levels does not mean that they will result in direct adverse health effects, but they can cause disorders in the human body. As the human health effects of these chemicals are inconsistent in epidemiologic research, it is still debatable whether such exposure affects human health (Kaiser, 2000; Snedeker, 2001). This section focuses on the human health disorders associated with organochlorine pesticides. To investigate their potential health risks upon exposure, biomonitoring of organochlorine pesticides in human serum is essential. General toxicological and environmental data on hazardous substances are compiled in toxicological profiles published by the Agency for Toxic Substances and Disease Registry (ATSDR). On the basis of these data, the agency derives chemical-specific minimal risk levels (MRLs) that assist in evaluating public health risks associated with exposure.

5.1 Thyroid hormonal imbalance

Most organochlorine pesticides and organochlorines may cause thyroid hormonal inactivity. Thyroid hormone is produced by the thyroid gland, and its major forms are thyroxine (T_4) and triiodothyronine (T_3). An imbalance of thyroid hormones can lead to various disorders. In 385 adult men, the serum concentrations of *p,p'*-DDE and HCB were found to be associated with thyroid hormone levels (Meeker et al., 2007). The former compound was associated with increased free thyroxine (T_4) and total triiodothyronine (T_3) levels, and inversely associated with thyroid-stimulating hormone (TSH). Hagmar et al. (2001) reported no associations between *p,p'*-DDE and thyroid hormones (Hagmar et al., 2001). Turyk et al. (2006) studied associations of total T_4 and TSH with organochlorines including *p,p'*-DDE using the National Health and Nutrition Examination Survey 1999–2000 data (Turyk et al., 2006). They found inverse associations of total T_4 with exposure to dioxin-like organochlorines, stronger associations in females, and a dose-dependent decrease in total T_4 with exposure to dioxin-like organochlorines at levels similar to those found in the general United States population. T_4 was positively associated with *p,p'*-DDE in all women but was negatively associated with this compound in men. Associations of thyroid-stimulating

hormone (TSH) with *p,p'*-DDE were insignificant and inconsistent (Turyk et al., 2007). A number of factors are related to the inconsistent human findings, including different detection methods as well as differences in age, gender, and other lifestyle factors.

5.2 Hormone-related cancers: breast and prostate cancers

Organochlorine pesticides may be associated with increased risk of hormone-related cancers including breast and prostate cancers. However, epidemiologic evidence is limited and inconsistent (Snedeker, 2001). Wolff et al. (1993) analyzed associations of serum *p,p'*-DDE concentrations with the risk of breast cancer (Wolff et al., 1993). During 1985–1991, cases ($n = 58$) and controls ($n = 171$) enrolled in the New York University Women's Health Study (NYUWHS), a prospective cohort study of hormones, diet, and cancer. The mean levels of *p,p'*-DDE were higher in patients with breast cancer than in control subjects. There were strong positive relationships between breast cancer risk and serum *p,p'*-DDE levels. On the other hand, the authors did not find associations of the risk of breast cancer with serum *p,p'*-DDE concentrations in a similar prospective investigation of the New York University Women's Health Study (NYUWHS) during the period 1987–1992 (Wolff et al., 2000). Cases ($n = 148$) and individually matched controls ($n = 295$) had similar serum levels of this compound, providing no evidence for an association of breast cancer risk with *p,p'*-DDE in blood. Recently, a case-control study was carried out to investigate the association between breast cancer risk and organochlorines in Japanese women, which is the first large-scale study of an Asian population (Itoh et al., 2009). The serum samples were collected from Nagano during 2001–2005 and consisted of 403 breast cancer cases and matched controls. Organochlorine pesticides and PCBs were measured by using isotope dilution gas chromatography–high resolution mass spectrometry. The serum concentrations of organochlorine pesticides were not associated with increased risk of breast cancer in the Japanese population.

Xu et al. (2010) used the National Health and Nutrition Examination Survey 1999–2004 data to examine associations between serum concentrations of organochlorine pesticides and prostate and breast cancers (Xu et al., 2010). After adjustment for other covariates, the serum concentrations of β -HCH, *trans*-nonachlor, and dieldrin were significantly associated with prostate cancer prevalence. However, there was no positive association between these serum concentrations and the risk of prevalent breast cancer. The Japan Public Health Center (JPHC) prospective study consisted of 14,203 men aged 40–69 years who participated from 1990 to 2005 (Sawada et al., 2010). From the cohort, 201 prostate cancer cases and two matched controls were compared for the median concentrations of organochlorine pesticides and PCBs. The odds ratios (ORs) and 95% confidence intervals (CIs) for prostate cancer were estimated in relation to serum levels of *p,p'*-DDT, HCB, β -HCH, *trans*-nonachlor, oxychlorane, and mirex. No significant differences in the median levels of these pesticides were found between the cases and the controls. Further, no statistically significant association with total prostate cancer was seen with any organochlorine, suggesting the lack of an overall association between prostate cancer and organochlorine pesticides at the levels measured in the Japan Public Health Center population.

5.3 Childhood developmental disorders

As organochlorine pesticides are present in both cord blood and breast milk, fetuses and infants can be exposed to these chemicals during the prenatal and postnatal periods. Fetuses

and infants are more vulnerable to neurotoxic environmental chemicals even at very low-level exposure, which can affect brain development, decrease cognitive function, and result in development disorders in childhood. Lee et al. (2007a) investigated learning disability (LD) and attention deficit disorder (ADD) in children aged 4–15 years from the National Health and Nutrition Examination Survey 1999–2000 data (Lee et al., 2007a). Dioxins in children showed significant positive associations with learning disability (LD), but *p,p'*-DDE and *trans*-nonachlor were not associated with the prevalence of learning disability (LD). β -HCH was inversely associated with learning disability (LD) but not significantly. Sagiv et al. (2010) found higher risk for attention deficit hyperactivity disorder (ADHD) at higher levels of PCBs and *p,p'*-DDE in a longitudinal cohort study including 788 mother–infant pairs (Sagiv et al., 2010). The PCB and *p,p'*-DDE levels in cord serum were moderately associated with attention deficit hyperactivity disorder (ADHD) in children aged 7–11 years born in 1993–1998 in a PCB-contaminated area (ORs = 1.76). These results support the view that low-level prenatal organochlorine exposure is associated with attention deficit hyperactivity disorder (ADHD)-like behaviors in childhood. During 2001–2005, women of reproductive age in Mexico provided blood samples, and after birth, each child was checked for cognitive and psychomotor development. Among 244 mother–child pairs, *p,p'*-DDE levels in the first trimester of pregnancy were significantly and negatively associated with the psychomotor development index (PDI) of the children (Torres-Sánchez et al., 2007). No significant association was found between *p,p'*-DDE levels and childhood neurodevelopment during the second or third trimester. Torres-Sánchez et al. (2009) also reported that the associations between prenatal exposure to *p,p'*-DDE in cord serum and neurodevelopment disappeared after 12 months of infant age (Torres-Sánchez et al., 2009).

5.4 Diabetes

The prevalence of diabetes has been increasing globally over the past few decades (King et al., 1998). Recent epidemiologic studies have shown that background exposure to persistent organic pollutants, especially organochlorine pesticides, is strongly associated with type 2 diabetes. Lee et al. (2006) demonstrated a very strong relationship between the levels of persistent organic pollutants in serum, particularly oxychlordan and *trans*-nonachlor, and the risk of type 2 diabetes in the general American population by extensive analysis of the National Health and Nutrition Examination Survey 1999–2002 data (Lee et al., 2006). This association was higher in obese people than among the non-obese. The associations between the serum concentrations of organochlorine pesticides and the prevalence of diabetes were examined in the Mexican-American population (Cox et al., 2007) and Korean population (Son et al., 2010). Exposure of *p,p'*-DDE was related to the incidence of diabetes in a cohort of Great Lakes sport fish consumers from 1994 to 2005 (Turyk et al., 2009). Lee et al. (2010) also investigated whether several persistent organic pollutants predict the risk of type 2 diabetes within the Coronary Artery Risk Development in Young Adults (CARDIA) cohort (Lee et al., 2010). Some persistent organic pollutants, such as *trans*-nonachlor and highly chlorinated PCBs, were associated with the incidence of type 2 diabetes over an 18-year period, especially in obese people. Persistent organic pollutants showed strong associations at relatively low exposures, resulting in inverted U-shaped dose–response curves instead of the traditional dose–response relationship with diabetes.

Country	Year ^a	n	β -HCH	HCB	Oxy	TN	DDE	DDT	reference
Korea	2006	40	49	16.7	4.8	6.5	224	18.6	Kang et al., 2008
Korea	2006	40	44	18.3	8.3	20.2	379	23.8	Son et al., 2010
Japan	2000	80	93.2	<LOD	9.0	20.9	221	<LOD	Tsukino et al., 2006b
Japan	-	18	50	13.5	7.3	21.9	218	11	Takasuga et al., 2006
Japan	1999	23	28 ^b	7.8	60 ^c	NA	230 ^d	NA	Minh et al., 2005
Japan	1999	32	34 ^b	13	26 ^c	NA	220 ^d	NA	Minh et al., 2005
Japan	1999	22	34 ^b	9.8	28 ^c	NA	200 ^d	NA	Minh et al., 2005
Japan	1999	152	280	21.6	13.8	50	312	28	Masuda et al., 2005
Japan	02-03	32	26 ^b	16.0	1.2	7.0	93	2.4	Fukuta et al., 2005
Japan	01-05	405	64	27	8.6	23	370	9.9	Itoh et al., 2009
China	2005	26	12 ^b	39	NA	NA	600 ^d	NA	Bi et al., 2007
China	2005	21	39 ^b	31	NA	NA	2300 ^d	NA	Bi et al., 2007
China	96-98	250	5065	62.7	NA	NA	7635	309	Lee et al., 2007b
New Zealand	96-97	60 ^f	10.7	<LOD	<LOD	<LOD	919	<LOD	Bates et al., 2004
Sweden	96-97	205	51	65	13	23	497	NA	Glynn et al., 2003
Sweden	96-99	323	9	23	<LOD	5	88	<LOD	Glynn et al., 2007
UK	2003	154	12	11	NA	NA	100	2.9	Thomas et al., 2006
Spain	1998	682	NA	NA	NA	NA	118	<LOD	Zumbado et al., 2005
Spain	92-96	953	221	462	NA	NA	858	<LOD	Jakszyn et al., 2009
Belgium	1999	47 ^f	6.0 ^e	92.2	NA	NA	944.9	3.7	Koppen et al., 2002
Romania	2005	142	923	30		4.0	1975	339	Dirtu et al., 2006
Slovakia	2001	1009	48.6	690	NA	NA	2521	72.9	Petrik et al., 2006
Slovakia	2001	1038	44.0	639	NA	NA	1368	33.2	Petrik et al., 2006
USA	99-00	1702	<LOD	<LOD	<LOD	17.8	226	<LOD	CDC, 2005
USA	01-02	2307	<LOD	<LOD	11.1	17.9	250	<LOD	CDC, 2005
USA	03-04	1796	<LOD	14.9	10.3	14.8	203	<LOD	Patterson et al., 2009
USA	97-98	70	40	239	249	NA	1268	25	Rusiecki et al., 2008
USA	59-67	283	NA	NA	NA	NA	5200	1400	Bhatia et al., 2005
USA	63-67	399	NA	NA	NA	NA	5878	1611	James et al., 2002

^aYear of collection; ^bsum of four hexachlorocyclohexane isomers; ^csum of *o,p'*-DDT, *o,p'*-DDD, *o,p'*-DDE, *p,p'*-DDT, *p,p'*-DDD, and *p,p'*-DDE; ^e γ -HCH; ^fpooled serum
 NA, not available; LOD, limit of detection; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; Oxy, oxychlorodane; TN, *trans*-nonachlor; DDE, *p,p'*-dichlorodiphenyldichloroethylene; DDT, *p,p'*-dichlorodiphenyltrichloroethane

Table 2. The median organochlorine pesticide concentrations (ng/g lipid) in serum samples of different countries

6. Conclusion

Human biomonitoring data show that organochlorine pesticides are detectable in the body despite their ban a few decades ago. Nowadays, it is accepted that the general human population is exposed to background environmental levels of organochlorine pesticides. The exposure levels vary according to the region, population, and race, and have been decreasing over time. However, the current background concentrations of each these pesticides are still much higher than those of notorious organic pollutants such as PCDD/Fs, PCBs, and PBDEs. In addition, many epidemiologic studies have revealed that their current levels in the general human population are associated with several health disorders and may cause adverse effects on human health. In view of the number of factors related to the inconsistent human findings, including different detection and quantification methods, we recommend continuous biomonitoring study of organochlorine pesticides in human serum based on the analytical method using isotope dilution gas chromatography - high resolution mass spectrometry.

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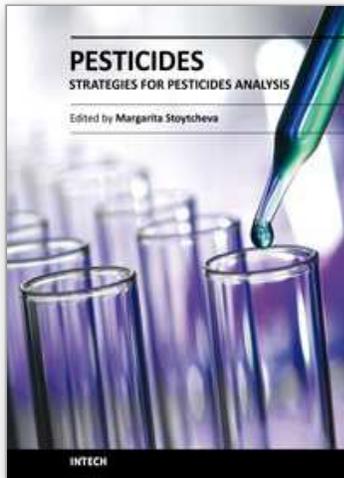
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This book provides recent information on various analytical procedures and techniques, representing strategies for reliability, specificity, selectivity and sensitivity improvements in pesticides analysis. The volume covers three main topics: current trends in sample preparation, selective and sensitive chromatographic detection and determination of pesticide residues in food and environmental samples, and the application of biological (immunoassays-and biosensors-based) methods in pesticides analysis as an alternative to the chromatographic methods for "in situ" and "on line" pesticides quantification. Intended as electronic edition, providing immediate "open access" to its content, the book is easy to follow and will be of interest to professionals involved in pesticides analysis.

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