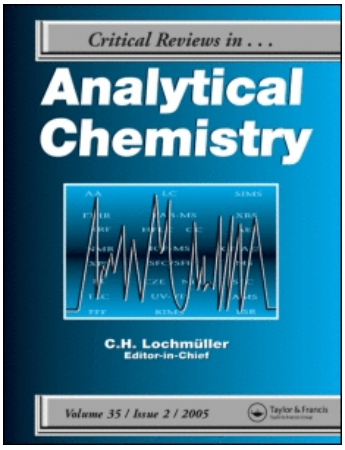

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An Analysis of Pesticides and Polychlorinated Biphenyls in Biological Samples and Foods

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Pesticides and polychlorinated biphenyls (PCBs) are found in various parts of the environment in quite small concentrations, but they accumulate and thus become a threat to the health and life of humans and animals. Humans, who are at the very top of the food chain, are the most vulnerable to the harmful effects of these compounds. An awareness of the dangers carried by ongoing pollution of the environment with these compounds, together with modern precautionary means should reduce the risk of poisoning among people—and the risk of irreversible damage being done to the natural environment—to an absolute minimum. The necessity to assay these compounds in various parts of the environment, and above all in food, is unquestionable as long as the awareness of these substances among people remains small. The variety of matrices in which pesticides and PCBs occur, and of their physical and chemical properties, requires the use of many methods for preparing samples for analyses and assaying. Selection of appropriate conditions of analysis determines the credibility of the end results.

Keywords biological samples, extraction, food, methods of final assays, pesticides, polychlorinated biphenyls, purification

Pollution of the environment with harmful chemical compounds is having an increasingly detrimental effect on the health of people and animals. The development of Earth's civilization not only should take into consideration the needs of the growing population, but also the necessity to maintain ecological balance in the world. Failing to fulfill this condition will cause deterioration of people's health and the extinction of many species of animals that are more sensitive to pollution than humans (1, 2).

Among harmful chemical compounds, pesticides and polychlorinated biphenyls (PCBs) are especially damaging due to their stability in the environment and their toxicity. Because of their chemical and physical properties, they are constantly present in many different parts of the environment. Alarming facts include the amazing stability of some pesticides and PCBs in the environment, their propagation in ecosystems on a worldwide scale, their infiltration into food, the occurrence of these compounds in the bodies of animals occupying the highest places in the food chain, and the proven high toxicity of these compounds (3).

In order to limit contact with these compounds, it is necessary to introduce standards for their highest allowable concentration in food, drinking water, and also in arable soil. For this purpose, it is necessary to use many methods allowing for assays of the concentration of pesticides and PCBs in various parts of the environment. Variety of matrix and physicochemical properties of these compounds leads to the need of applying a great deal of sample pretreatment for preparation before analysis and their determination. Analytical procedures consist of successive stages: sampling and sample preparation, extracting analytes from a matrix, extract purification before analysis, and proper final determination.

SAMPLING AND SAMPLE PREPARATION FOR ANALYSIS

Selection of the appropriate method of assaying pesticides and PCBs depends on many factors. The problem with analysis of biological material is the immense variety and complexity of matrices in which pesticides and PCBs are assayed.

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Collection of samples of biological material involves many difficulties which is why, when taking a sample for analysis, one should consideration take into mainly the purpose of the examination, the concentration of analytes, the effectiveness of extraction and enrichment, and the sensitivity and selectivity of the final analysis method. Many biological samples are naturally nonhomogeneous, making it difficult to obtain a representative sample. Samples taken must be kept in appropriate conditions: frozen (−20°C) and in a dark place. Failure to meet these requirements, especially with regard to food and biological materials, may result in decomposition of the sample material and the loss of the analytes. The next step is to dry the sample. Drying may take place at a high temperature (in a drier), at room temperature (air-dried samples), and through the addition of a drying agent (e.g., anhydrous sodium or magnesium sulfate). An increasingly popular method of removing water from solid materials is freeze-dring. The samples to be freeze-dried are frozen to a temperature of −20°C. Then at a reduced pressure and temperature, water passes to the gaseous state, and in this way—omitting the liquid state—it is removed from the sample. Before analysis solid samples are broken up in special devices, or powdered in grinders or mortars (hard materials) (4). They also are homogenized, usually with the addition of an appropriate solvent, in homogenizers. A sample prepared in this manner is subject to further stages of analysis.

METHODS OF ISOLATION OF PESTICIDES AND PCBs

The basic methods used for isolating pesticides and PCBs from biological samples include: liquid-solid extraction (LSE), solid-phase extraction (SPE), matrix solid-phase dispersion extraction (MSPDE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE), solid-phase micro extraction (SPME), Soxhlet extraction, and microwave-assisted extraction (MAE). When choosing an extraction method, one should take into consideration the chemical and physical properties of the analytes and the type of matrix in which they are present.

Liquid-Solid Extraction

Extraction in a liquid-solid setup is based on the distribution ratio of the analyte between solid and fluid. When assaying several compounds at the same time, the solvent used for extraction should efficiently isolate different chemical compounds belonging to various classes and coming from samples containing large amounts of water, fats, sugars, and other substances. The selection of an appropriate solvent for extraction is determined by the conditions of extraction; in other words, we want to obtain a maximum of analytes and a minimum of coextraction of interfering substances. The most commonly used solvents for extracting pesticides and PCBs are acetone and acetonitrile, as they mix well with water and thus easily allow for analytes to pass to the organic phase and for the removal of any water that may be contained in the sample. Other organic solvents, that do not mix with water, can also be used for extraction. These too can effectively and selectively extract pesticides, for example, kerosene ether used mainly for extracting nonpolar pesticides and PCBs, dichloromethane for isolating polar pesticides, and hexane for extracting pesticides and PCBs from fatty matrices (meat, milk, fish livers) (5). In order to achieve greater selectivity of extraction, we also use mixtures of solvents, for example, a mixture of acetonitrile and acetone or hexane for extracting organophosphorous pesticides from oil (6), a mixture of ethyl acetate and methyl alcohol for isolating pesticides from muscle tissue (7), a mixture of chloroform and methyl alcohol for extracting PCBs from fish flesh (8), and a mixture of acetone and hexane for extracting PCBs from butter (9). A certain modification of extraction using a solvent is ultrasound-assisted extraction, which applies an additional source of energy, helping to release the analytes from the sample (2).

Solid-Phase Extraction

SPE is based on adsorption of analytes onto solid sorbents. This is a technique commonly used for enriching analytes from liquid and gaseous matrices. Many food matrices have been cleaned up by SPE for determination of such a large number of analytes.

The SPE extraction or purification is performed in four steps:

- conditioning (the functional groups of the sorbent bed are solvated in order to make them to interact with the sample),
- retention (the analytes are bound to the bed surface),
- selective washing (undesired species are removed), and
- elution (the analytes are desorbed and collected for analysis).

The selection of the appropriate sorbent depends on the mechanism of interactions between the sorbent and the analytes. Understanding that mechanism, in turn, depends on the knowledge of the hydrophobic, polar, and inorganic properties of both the solute and the sorbent. Many types of sorbent, such as alumina, magnesium silicate, and graphitized carbon, are commercially available, but the most common material is silica because it is reactive enough to permit its surface to be modified by chemical reaction and yet stable enough to allow its use with a wide range of solutions. Polymer-based sorbent beds are very popular too, and lately their molecular imprinting holds out considerable promise to complement the repertoire of SPE materials for food analysis (10, 11). Sorbents fall into three general classes: nonpolar, polar, and ion exchange. Their activity is dependent on the properties of the bonded phase and if any active site not and capped on the sorbent. The choice of the sorbent is dependent on the food matrix, analytes of interest, and their interferents.

The SPE method is simple, effective, and econimical. Its advantage, compared to classical liquid-liquid extraction, is the elimination of the stage of division into two liquids and the resultant problem with an emulsion appearing. A new innovation

has been the introduction of a disk format which, in comparison with a packed cartridge, offers larger flow area and lower bed mass (11).

A more selective approach employs immunoaffinity chromatography (IAC) in which antibodies are coupled to the solid phase leading to specific retention on immunosorbent SPE cartridges. The use of immunosorbent SPE cartridges has considerably simplified the analysis, for example, of triazines in carrots, celery, corn, grapes, onions, potatoes, and strawberries (10).

Matrix Solid-Phase Dispersion Extraction

Extraction from the solid phase is a new technique during which extraction, purification, and fractioning take place—all in one process. A homogenized sample is mixed with a solid sorbent, for example, silica gel, Florisil, aluminum oxide, or sorbents with chemically bound phases containing C_{18}, C_8, C_2, and CN type function groups. Mixtures, such as silica gel and charcoal are used as solid sorbents. The layer of sorbent with adsorbed analytes is placed in a column, on a layer of anhydrous sodium sulfate (to capture the water contained in the sample). Next, they are washed out with an appropriately chosen solvent that ensures a good separation of the analytes.

A certain modification of this method is the application of a series of columns (by connecting an additional column with a sorbent deposit). Eluate, after passing through such a setup, can be immediately analyzed, without further purification. Such an assembly was used to extract analytes from samples of cauliflower and cabbage. Satisfactory results were achieved using a deposit of silica gel as a solid sorbent for extraction. Washing analytes out of the column was performed using a mixture of dichloromethane and acetone and, subsequently, the eluate was purified on several selected deposits: alumina, silica gel, Florisil, charcoal, and combinations of these. The applied bed of Florisil with charcoal, used for purifying the extract, allowed us to obtain the greatest amount of analytes compared to purification on other deposits (10, 12). The SPE method was also used to assay organophosphorous pesticides in milk. Diatomaceous material was used as a sorbent, and it was placed in disposable cartridges. A mixture of high-grade kerosene and methyl cyanide (MeCN) or acetonitrile and ethyl alcohol was used as a solvent (10, 13). A similar procedure was applied to isolate and assay pesticides in soybean oil. The sample was mixed with Extrelut-3 sorbent and extracted with acetonitrile. The remaining oil in the extract was removed with high-performance size, exclusion chromatography (SEC) on a mini- and macrocolumn. The two techniques used in series allowed a better removal of fat, a greater input of sample, and a lower consumption of solvent compared to the sole SEC on macrocolumns, and a lower limit of determination compared to the sole SEC on minicolumns (14). MSPDE is a technique in which sample extraction and clean-up are carried out in the same step with good recovery and reproducibility, reducing the analysis time and the amount of solvent employed. The three main advantages of MSPDE are: it permits rapid sample turnover, enhancing access to timely data on residue levels present in the sample; it requires a small sample size; and it decreases considerably the amount of solvent used compared to the classical methods and thus, in turn, decreases environmental contamination and increases worker safety.

Supercritical Fluid Extraction

SFE is a commonly used extraction technique because of the small amounts of solvents used. This technique, in most cases, brings a high degree of extraction selectivity. The technique employs a liquid in supercritical state as the extracting medium. As a result of the solvent force of the extracting medium, selective extraction of particular components is possible. The supercritical-state fluid has properties of both a liquid and a gas, which means that it can pass through the matrix like a gas does, but it is also a solvent like a liquid which allows it to penetrate the matrix easily and release the analytes into the gaseous phase. The effectiveness of the process can be adjusted by changing the pressure and temperature. At constant temperature extraction is more efficient at lower pressures for lighter and less polar compounds, while at higher pressures larger and more polar molecules are extracted (10). Carbon dioxide is used as the supercritical-state liquid, and it is modified with an addition of methyl alcohol or water, thereby extending the range of applications of the method (e.g., for extracting highly polar pesticides and their metabolites). Less commonly used modifiers are hexane and methyl cyanide (15). SFE has been used to extract pesticides from various matrices (e.g., wheat or corn grain) using carbon dioxide as the supercritical-state liquid (16), and to extract pesticides from chicken eggs using carbon dioxide modified with methyl alcohol as the extracting medium (17).

There exists the possibility to join supercritical liquid extraction with sorption on a solid sorbent to purify the extract. This consists of placing the sorbent in an extraction cell and performing extraction simultaneously with purification. The method is finding ever broader use, as it does not lead to a degradation of the extracted compounds; it is fast, it is not labor consuming, it does not require the use of toxic solvents, and it is selective. An unquestionable advantage of this method is also that the extract obtained is very pure and does not require further purification. Such a solution has been used to extract and purify extracts of pesticides and PCBs obtained from clam flesh. Prior to analysis, the flesh was freeze-dried, homogenized, and placed in an extraction cell with a deposit of Florisil, where extraction was performed. Other sorbents were also used, such as silica gel with C_{18} phase and neutral alumina, for example, for purifying extracts of pesticides and PCBs from fish flesh (18). It is also possible to extract using supercritical-state fluid with simultaneous purification, by using an automated SFE system linked with C_{18} phase. In this case, carbon dioxide modified with methyl cyanide was used as an extraction medium. Application of such a technique for extraction of pestcides and PCBs from matrices with a high fat content yielded very satisfactory results (19).

Accelerated Solvent Extraction

ASE is a most often used technique for isolation of analytes from solid and aqueous samples. The process consists of using the extraction properties of solvents at high pressure and high temperature. Nearly all available organic solvents or their mixtures are used as an extraction medium in this method. A higher pressure during the process allows it to maintain the hot solvent in liquid phase above its boiling point. In this condition, its physical and chemical properties are more beneficial to the extraction process and ensure a more desirable course of the process. Low viscosity of the solvent at a raised temperature causes a better penetration of the sample, which in turn improves the efficiency of the extraction, while higher coefficients of diffusion and high solvent power improve the kinetics of the reaction (20).

The sample meant for extraction is usually placed in a drying medium, such as anhydrous sodium sulfate or diatomaceous earth, which bind the water contained in the sample. The best way to dry the sample is to freeze-dry it to solid state. Apparatus for accelerated extraction using a solvent called ASE 200, available from the Dionex Company, consists of extraction cells (volumes: 11, 22, or 33 mL) in which the sample is placed. In this method of extraction, a solvent and a gas (nitrogen) are fed into the extraction cell through special tubes. In order to secure the apparatus from contamination, the tops of the extraction cells are equipped with special sinters. Furthermore, paper filters are placed on the cell bottoms. The cell is heated in a furnace until the sample reaches a condition of thermal equilibrium. Pressure is also controlled—this is the stage of statical extraction. Following this stage, the extracts are moved to special receivers. The sample material remaining in the cell is washed with a portion of pure solvent and then with a stream of nitrogen or air, in order to clear out any remaining solvent. The ASE technique can be used for extraction of a wide range of analytes from various solid matrices. ASE has been used for extraction of pesticides and PCBs from matrices with a high fat content, such as poultry, eggs, and fish meat. Samples were homogenized, dried with anhydrous sodium sulfate, and subjected to accelerated extraction with hexane. Following extraction, the secreted fat solution was dried with anhydrous sodium sulfate and assayed for fat content by gravimetric analysis, while the extract was purified and analyzed using chromatographic techniques (21, 22).

The advantage of ASE is that it can be used to extract perishable analytes in high temperatures due to the fact that the process takes place very rapidly and practically without any oxygen. Other advantages are: a considerable reduction of the amount of solvents used, personnel time saved, and the ease with which samples can be prepared. The ASE system can be fully automated, allowing it to perform the particular stages of extraction in an identical manner and thus allowing it to avoid mistakes at this stage of analytical procedures. The main limitations of this method are: the high cost of the apparatus and the method's lack of selectivity, as well as the necessity to purify the extract.

Solid-Phase Microextraction

SPME is a fast and simple method, not requiring organic solvents and it can be fully automated. This technique is a certain variation of SPE, one of the differences being that the sorbent is modified and is spread onto a thin glass or quartz fiber. Such a placing of the sorbent layer takes advantage of the cylindrical shape of the fiber surface. This facilitates exchange of mass during enrichment and releasing of the arrested compounds and eliminates problems connected with clogging the sorbent bed. The process is controlled by the diffusion of analytes through the static layer surrounding the fiber. Diffusion into the polymeric film is aided by sample agitation which helps to establish equilibrium. Equilibration time is dependent on the thickness and type of the coating and distribution constant of the analytes. The sensitivity of the extraction depends on the affinity of analytes for the coating and its capacity (11).

Analysis using the microextraction technique to the stationary phase consists of two stages. The first stage is division of the organic compounds between the stationary phase set on the fiber, and the matrix—this is the adsorption stage. The second stage is thermal desorption in a hot injector. The high temperature in the injector causes a considerable change in the partition coefficient of compounds trapped on the stationary phase toward desorption to the gaseous phase (carrier gas). The analyte molecules released in this way are carried away by the carrier gas and taken to the chromatographic column, where they are separated and quantitatively assayed. Both steps must be optimized for a successful procedure. Factors influencing the extraction step include fiber type, extraction time, ionic strength, sample pH, extraction temperature, desorption time, focusing oven temperature, and solvent employed and its volume.

The most commonly used sorbents are: polydimethylosiloxan (PDMS), polyacryl (PA), and various mixtures of these (e.g., polydimethylosiloxan/polydivinylobenzene (PDMS/DVB), Carbowax, and Carboxen (23)). Microextraction to the stationary phase brings extraction and enrichment of the analytes together in one stage. An advantage of this method is the complete elimination of solvents. SPME can be used on a wide variety of samples, both liquid and solid food. The SPME technique is used to analyze such materials as tobacco, herbs, spices, juices, alcoholic beverages, honey, and more recently fruits, fruit juices, wine, more also meat, fish, honey, milk, and eggs (10, 11, 24, 25).

Soxhlet Extraction

Extraction in the Soxhlet extractor is a technique used for isolating analytes from solid matrices. This is a type of continuous extraction. The sample meant for extraction is placed in the apparatus where a hot condensate, of a solvent distilling in a closed circuit, extracts the organic substances from the sample. Owing to the closed circuit and distilling of the solvent, the sample may be extracted many times with fresh portions of solvent, and the sample need not be large. The size of the system can

vary, but the most common configurations use about 100 mL of solvent and 5–50 g of solid sample with extractions lasting from 4 to 18 h. This type of extraction is quite a slow process. However, it is not a very labor-consuming one, as it does not require continuous supervision. Automation allows for acceleration of the process and reduction of the amount of reagents used (2). Another advantage of this method is that the sample need not be homogenized prior to extraction. Extraction in the Soxhlet extractor has been used to isolate pesticides and PCBs from environmental samples with high lipid content. Extraction of pesticides has been performed on samples of sea animals, such as oysters and shrimp. The samples were freeze-dried and extracted in a Soxhlet extractor with a specified volume of hexane for 16 h (26). The technique was also used to isolate PCBs from sea fish tissues (27) and animal milk (28). In all the cases, an acetone/hexane mixture was used as an extracting medium. Compared to extraction with a solvent by shaking, extraction in a Soxhlet extractor allows one to obtain a better isolation of analytes from the same samples.

Microwave-Assisted Extraction

MAE applies the effect of adsorption of microwave energy by particles of chemical compounds. A Teflon or quartz bomb is used for extraction and the solvent for extraction of pesticides and PCBs is usually dichloromethane or an acetone/hexane mixture. There are two ways of performing extraction. One of them consists of using a solvent that absorbs microwaves (with a high dielectric constant). Microwaves cause the solvent to heat over the boiling point, and a hot solvent enables rapid extraction of the analytes from the matrix. The second method of performing extraction is to use a solvent that does not absorb microwaves (with a low dielectric constant). Subjected to microwaves, the solvent does not heat, as it does not absorb microwave energy. The compounds contained in the sample (e.g., water and other compounds with a high dielectric constant) absorb microwaves and release their heat to the cool solvent, which is selected in such a manner as to be able to dissolve the sample (2). For example, in the case of n-hexane, the addition of a microwave transformer is necessary, because it has a relatively low dielectric constant and cannot be directly heated using microwave energy. The microwave transformer is not needed, however, in the case of solvents with a high dielectric constant such as ethyl acetate (20).

MAE can be used on thermally unstable compounds (e.g., organochlorine pesticides). Its advantage is a quick and effective extraction of analytes from the matrix and a low consumption of solvents. MAE together with purification using gel chromatography and additional purification on a silica gel deposit was used to isolate PCBs from the fat tissue of a seal and from cod liver (a matrix with a high fat content approximately 50% and water content approximately 30%). MAE was performed by both methods: using n-hexane and an ethyl acetate/cyclohexane mixture. BioBeads SX-3 were used as a deposit for purification in gel chromatography. Comparing both methods, it was noted that extraction using hexane is better for samples with a higher fat content, while the ethyl acetate/cyclohexane mixture is better for samples with a lower fat content (29).

A recently developed technique for extraction of organic compounds is focused microwave-assisted extraction (FMV). This novel technique is rapid (10 min) and uses a reduced volume of solvent (30 mL). The extraction proceeds at atmospheric pressure and requires only basic manipulation. This technique can be used for extraction of organic compounds from food and plants (e.g., mussel tissue, cod liver). FMW is a good alternative to the classical extraction technique and is suitable for environmental studies (30).

PURIFICATION METHODS (CLEAN-UP)

The extract purification process is an important stage of the entire analytical procedure. The examined matrices contain various kinds of interfering compounds (water, fats, sugars, chlorophyll, and many others), which are extracted together with analytes and must be removed if the analysis is to be precise. Purification consists of fractioning the extract using adsorption chromatography, gel chromatography, or column chromatography.

Adsorption Chromatography

The most commonly used method of purification is adsorption chromatography applying the SPE technique. This technique consists of adsorption of analytes and interfering substances, on a solid sorbent, followed by elution of the analytes (using appropriate solvents) from the substrate, and arresting the interfering substances. Nowadays, analysts use chromatographic columns that are factory-made and filled with modified silica gel, Florisil, or alumina. These fillings are used for purifying extracts from lipid fractions. In the case of organonitrate and organophosphate pesticides, a common method of purification and extraction is using column with a bed of silica gel modified with C_{18} phase or, in the case of isolating organonitrate pesticides, modifed with $-SO_3H$ phase and a Florisil bed and various solvents for elution (29, 31). Purification using the SPE technique is widely applied in every analytical procedure if it is necessary to remove interfering substances. In some cases, purification in columns can take a long time due to a limited flow of liquid through the deposit. For this reason, instead of adsorbents, analysts often use thin disks with diameters considerably greater than that of the column. These may be Speedisks and Speedisk columns available from J. T. Baker Company (32, 33). The bed of sorbent is located between two disks made from Teflon fiber, which prevent clogging the deposit by particles contained in the sample. Speedisk columns are disposable, ultraclean, polypropylene columns prepacked with high-performance microparticles. Featuring a unique laminar configuration, Speedisk columns operate with smaller solvent volumes and have higher capacity per milligram sorbent than conventional SPE columns. The Speedisk design shortens run

times, increases capacity, and may eliminate prefiltration and evaporation steps. Speedisks are used in enriching and purifying samples of large volume and with a high content of suspended matter.

Gel Permeation Chromatography

Gel chromatography is the most commonly used technique for purifying biological samples, especially with a high fat content. The mechanism of gel chromatography consists of excluding particles of different dimensions. Most pesticides have a molecular mass within the range of 200 to 400 while lipids, which must be separated from the pesticides, have a molecular mass of around 600 to 1500. Larger molecules are stopped in the deposit, smaller ones are washed out. Columns used in gel chromatography are usually filled with copolymers of styrene and divinylbenzene, carrying the commercial name BioBeads SX-2 or SX-3. These deposits have a wide range of pore diameters. In the case of fillings with large pores (molecular mass around 1000 to 2000), the separating mechanism is both exclusion and adsorption. The mobile phases used are, for example, cyclohexane or combinations of solvents with various elution force (CH_2Cl_2/hexane, CH_2Cl_2/cyclohexane, toluene/ethyl acetate, ethyl acetate/cyclohexane, and acetone/cyclohexane). In the case of fillings with small pores (molecular mass around 400) in combination with strongly polar solvents (e.g., tetrahydrofurane or dimethyloformamide), the exclusion mechanism is dominant. Analytes arrested in the deposit are washed out with a solvent selected to fit the properties of the analytes, for example, a cyclohexane/vinyl acetate mixture (2). Another commonly used filling is porous graphitic carbon (PGC). Columns filled with PGC have been used to purify extracts of environmental samples (soil, water) from lipid fractions (34). PGC is being used more and more frequently as the purification stage of extracts following extraction to the solid phase. For example, to remove fat from an extract for analysis of pesticides, a PGC column was used, filled with polymer BioBeads SX-3 with grain of appropriate size, and the mobile phase was a cyclohexane/ethyl acetate mixture (35). Gel chromatography was also used to purify extracts obtained from samples of vegetables and fruit (apples, grapes, melon, peaches, strawberries, carrot, spinach, tomatoes, etc.). A deposit of Envirosep SX-3 (a styrene-divinylbenzene copolymer) was used as the filling of the PGC column, while the mobile phase consisted of an ethyl acetate/cyclohexane mixture. Separation and assaying were performed in a chromatograph with an electron capture detector and mass spectrometer (36). Gel deposits are used to purify extracts of nearly all pesticides, yielding extracts ready for analysis. The advantages of gel chromatography include the possibility to automate the process and a long technical lifetime of the columns, which can be used for many months without any visible change in retention volume or their ability to separate.

A certain modification allowing one to eliminate errors connected with sample preparation is the connected system of purifying extracts using gel chromatography and final assaying of pesticides using a gas chromatograph with an NPD detector. Such an assembly was used to assay, for example, pesticides and PCBs in olive oil. The column contained a PLGel deposit, while the mobile phase was an ethyl acetate and cyclohexane mixture with a 6% solution of n-decane. This method allows for sufficient separation and assaying of organophosphorous pesticides from fat (37, 38).

For purification of extracts from samples with a high fat content, analysts use a new technique. The innovation is the high-capacity disposable silica (HCDS) column, filled with various forms of silica (e.g., 28 g of acid silica, 16 g of basic silica, and 6 g of neutral silica). HCDS columns were the first to make contact with a high-fat sample (above 4 g) and arrest the fat on the deposit. HCDS columns are connected with classical Power Prep column assemblies. The system is automated. This technical solution is used to purify extracts from samples with high lipid content, such as poultry, fish, and eggs (28).

Reversed-Phase Liquid Chromatography

Reversed-phase liquid chromatography (RPLC) utilizes solubility properties of the sample in very much the same way as the organic chemist does when he or she purifies a crude sample by partitioning it between a hydrophilic and a lipophilic solvent in a separatory funnel. The partition of the sample components between the two phases will depend on their respective solubility characteristics. Less hydrophobic components will end up primarily in the hydrophilic phase, while more hydrophobic ones will be found in the lipophilic phase. In a way, one can say that the whole process depends on the extractive power of the hydrophilic phase. This can be affected by the addition of an organic solvent which is soluble in the hydrophilic phase.

A high concentration of the organic solvent will increase the extractant power for hydrophobic compounds. In RPLC, silica particles covered with chemically bonded hydrocarbon chains represent the lipophilic phase (C_2 to C_{18}), while an aqueous mixture of an organic solvent surrounding the particle represents the hydrophilic phase. When a sample component passes through an RPLC column the partitioning mechanism operates continuously. Depending on the extractive power of the eluent, a greater or lesser part of the sample component will be retained reversibly by the lipid layer of the particles, in this case, called the stationary phase. The larger the fraction retained in the lipid layer, the slower the sample component will move down the column. Hydrophilic compounds will always move faster than hydrophobic ones, since the mobile phase is always more hydrophilic than the stationary phase.

Membrane Separation

Nowadays the most often used technique is membrane separation. The selective nature of membranes has made them a unique alternative to solvent extraction for sample clean-up, especially if coupled with chromatographic techniques. The

TABLE 1
Methods of Sample Extraction and Clean-Up (10)

Technique	Extraction	Clean-up	Characteristics
LLE	+	+	Foods, liquids, crops, plant material. High consumption of organic solvents, risk of emulsion creation, universal.
SPE			
Cartridge	+	−	Foods, liquids, crops, plant material.
Membrane extraction disc			No emulsion, low consumption of organic solvents,
GCB			automated, universal.
SPME	+	−	Foods, liquids, crops. No organic solvents, automated, universal.
MSPDE	+	+	Plant material, food, liquids, feed. No emulsions, direct on-line clean-up, universal.
SFE	+	+	Cereals, plant materials. Use ecologically nontoxic fluid (CO_2). Optimization of modifier, automated, expensive instrumentation, small extractions.
ASE	+	−	Foods, crops, plant material. Low use of solvents, automated, expensive instrumentation.
MAE	+	−	Foods, crops, plant material.
Column AC			Foods, crops, plant material, fatty foods.
Florisil column	+	−	
Alumina column	+	−	
Silica gel column	+	−	
PGC column	+	−	Samples with high content of fat, oil. Automated, economical, universal.
Soxhlet extraction	+	−	Foods, crops, plant material, fatty foods. Time consuming, high consumption of organic solvents.

+, Main application; −, secondary application.

Abbreviations: LLE, liquid-liquid extraction; SPE, solid-phase extraction; SPME, solid-phase micro extraction; MSPDE, matrix solid-phase dispersion extraction; SFE, supercritical fluid extraction; ASE, accelerated solvent extraction; MAE, microwave-assisted extraction; column AC, ; PGC, porous graphitic carbon.

relative sizes of different molecules largely determine the permeation selectivity of a membrane. The process is the result of differences in the transport rates of the species through the membrane interface: The separation is achieved when some species are transported to a greater extent than others. The forces able to generate transport through membranes are directly related to differences in pressure micro- and ultrafiltration) in concentration (dialysis) or in electrical potential (electrodialysis).

Membranes are usually made of synthetic materials although natural substances, such as cellulose, or inorganic materials, such as glass fibers or alumina, are also used (11). In food analysis liquid samples can be directly dialysed because the process does not suffer from the presence of particulate matter or macrocompounds such as proteins and lipids. The solid samples require the addition of water and homogenization before dialysis. Methods of sample extraction and clean-up are summarized in Table 1 (10).

FINAL DETERMINATION

Selection of the appropriate chromatographic technique is determined by the properties of the examined components. The main methods of final assay are: high-performance liquid chromatography (HPLC) and gas chromatography (GC), as well as immunological tests and increasingly popular biological methods. Most frequent, however, is the capillary GC using specific detectors for a given group of compounds. Separation is performed in a nonpolar column or a weakly polar column, while quantitative analysis is performed through the addition of a standard. The operating conditions of a chromatograph are selected depending on the properties of the analyzed group of compounds.

The variety of the analyzed compounds may make the resolution of capillary columns insufficient. This problem may be overcome by increasing the resolution of the chromatographic assembly, and such an oportunity is offered by two-dimensional

GC (2). The principle of this chromatography lies in using two chromatographic columns. The analytes, following preliminnary separation in the first column, are held in a cryogenic trap and next, after release, they pass to the second chromatographic column, where they are separated and assayed by an appropriate detector.

The most frequently used apparatus for assaying pesticides and PCBs is a gas chromatograph with a suitable detector. In choosing the detector, it is important to note the range of concentrations of analytes in the sample, the composition of the matrix, and, if necessary, the possibility to recover the analyte at the outlet of the column. Among detectors used for assaying pesticides and PCBs, the most common are: mass spectrometer detectors (MS), electron capture detectors (ECD), and thermionic detectors (NPD) (39). Other detectors used in GC are: electrolytic conductivity detectors (ELCD) and flame photometric detectors (FPD). Data obtained from analysis using a gas chromatograph coupled with a mass spectrometer, can be supplemented by an atomic emission detector (AED).

A widely used technique, especially for assaying compounds that have low volatility or are thermally unstable, is HPLC (40). In liquid chromatography, we use columns of larger diameters than in GC and they are filled. The mobile phase in liquid chromatography is a solvent, which mixes with the solvent of the sample. Selection of the stationary phase is determined by the necessity to ensure an interaction between the assayed components of the sample and the stationary phase. Some compounds can be assayed using columns with a polar stationary phase (silica gel) as well as with a nonpolar stationary phase (e.g., LiChrosorb RP_{18}).

Selection of the detector, and therefore of the sensitivity of the method, depends on the size of the sample. With small volumes of samples and low concentration of the analyte, detectors with a high sensitivity are needed. Among the wide range of detectors, fluorescent detectors, electrochemical detectors, absorption detectors operating in UV-diode array detectors (DAD) have the highest sensitivity and they are most often used (41). Now in HPLC most often a mass spectrometry (MS) detector is used.

An innovation in chromatography is to combine the GC and HPLC methods, forming a hybrid method, with fluid in supercritical state: supercritical fluid chromatography (SFC). This method of chromatography is regarded a very valuable one. Its advantage over other techniques is its universality in separating compounds through the addition of modifiers and selection of the stationary phase, as well as the possibility to use various detectors (used both in LC and GC) (38). There also exists the possibility to combine the SFE extraction method with SFC, which allows us to selectively extract analytes with small amounts of an organic solvent and deliver them into a column, omitting the dosage stage (42).

A relatively new technique used for determination of pesticides and PCBs in various matrices are the immunosorbent methods. Immunoassays are analytical methods making use of a specific interaction between the antigen, which is the target molecule, and an antibody that is sensitive to this target molecule. The immunosorbent methods include: enzyme-linked immunosorbent assays (ELISA), fluoroimmunoassay, and bioassay.

ELISA are the most extensively studied types of immunoassay with their application in pesticide and PCBs residue monitoring. Immunoassays supplement traditional analysis methods because they offer the possibility of highly sensitive, relatively rapid, and cost-effective measurements. The ELISA method was used for analysis PCBs in fish samples. Fluoroimmunoassay distinct improvement of immunoassay sensitivity was obtained by fluorescence labeling using lanthanide chelates. These compounds have the unique characteristics of a wide Stokes shift, which is the difference of the excitation and the emission wavelength, and a long-lasting fluorescence. Bioassays are analytical methods that measure the interaction of chemicals with cells or an organism. This technique is capable of detecting ceratin dioxins and dioxin-like PCB compounds (45).

SUMMARY

A problem connected with application of pesticides and PCBs is the necessity to monitor their residues in the environment, especially in food. Analysis of pesticides and PCBs in food is connected, on the one hand, with the specific character of biological material while, on the other, with the constant lowering by the European Union, of the so-called maximum allowable content of residues of these compounds in food products, especially in vegetables and fruit. This requires the development of new methods for assaying these compounds in various, often complex matrices. A versatile isolation and purification technique for general use with analyte and matrix is not known; however, it is desirable to combine the simple analyte isolation from a biological matrix with the effective purification technique of a multiresidual and multimatrix character.

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