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Determination of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls and chlorine-containing pesticides in the environment

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ABSTRACT. Literature material has been correlated on methods of determining ecologically particularly dangerous polychlorinated compounds (dioxins, furans and biphenyls) and chlorine-containing pesticides and phenols in the environment, viz. in air, natural waters, soil, sludges and various tissues of biological organisms (including man). All stages of the analysis are reviewed, i.e. selection and storage of samples, methods of extraction and derivatisation, means of purification of organic extracts, gas chromatographic and gas chromatography-mass spectrometry methods of separating and estimating individual isomers and compounds and their detection limits.

The bibliography contains 164 references.

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I. Introduction

Polychlorinated organic compounds (dioxins, furans, biphenyls) and chlorine-containing insecticides, herbicides and phenols have currently become a global ecological hazard because of their fairly high toxicity for mammals, including man, their persistence and their universal distribution both in the environment and in foodstuffs. Particular danger is presented by the 12 isomers of dioxin and furan containing 4–6 atoms of chlorine, of which 4 must be in positions 2, 3, 7 and 8 , and 13 isomers of biphenyl containing 4–7 chlorine atoms of which 4 must be in positions 4,4′ and/or 3,3′ and 5,5′. These isomers are very toxic compounds, they are synthesised by man and reliable methods have been developed for their quantitative determination at the ppt level (ng kg^{-1}). The limits of detection of chlorine-containing pesticides and their metabolites have been reduced significantly. The metabolites proved to be either precursors of xenobiotics of the dioxin type or synergistic with them.

The review is devoted to an analysis of the work of the last 5–7 years on the methods of determining quantitatively polychlorinated compounds of the types indicated. Special attention has been allotted to the isomer-specific determination of dioxins, furans and biphenyls.

II. Insecticides

Chlorine-containing insecticides are applied in the struggle against harmful insects. In this section most attention will be paid to the determination of 4,4′-dichlorodiphenyltrichloromethylmethane (DDT), its metabolites 4,4′-dichlorodiphenyldichloromethylmethane (DDD) and 4,4′-dichlorodiphenylchloroethylene (DDE) and also of the isomers of hexachlorocyclohexane (HCCH).

1. Selection and storage of test samples

Test samples of natural waters are taken with glass, teflon or metallic samplers, from vessels or bathometers. The volume of sample taken may vary from 4–25 litres depending on the assumed concentration of insectide.[1–4] It is not subjected to conservation and storage in view of the possible loss of insecticide as a result of adsorption onto vessel walls. Consequently it is necessary to extract the samples taken with an organic solvent, frequently hexane, within 2 hours. The organic extracts are stored in bottles with ground-glass stoppers. It is best to store them in the frozen state.[3] After storage at room temperature analysis must be carried out no later than 3 months after taking the sample.[1,2]

Sludges are selected with various hollow tubular samplers to avoid mixing when taking samples. Usually 10–15 cm upper layer of sediments is taken.[4–6] They are stored in glass jars at 4 °C or at −10 to −15 °C.[4,6] At room temperature samples may be preserved by acidification with hydrochloric acid to pH 2 and stored in that form for a month.[6]

Soils are sampled with an auger or a scoop and samples are mixed. They are air-dried in the laboratory or in the open air. They are stored in the dry state in small linen bags, and if naturally moist in the refrigerator.[7]

Test samples of air are always concentrated by passing them into liquid[8] or solid[9] adsorbents. The volumes of air may therefore amount to thousands of cubic metres.[9]

Biological samples are stored in the frozen state at −10 to −78 °C.[4,5,10–16] Only freshly taken samples must be frozen.

2. Extraction

Insecticides are usually extracted from test samples of natural waters with hexane[1–3,17,18] or dichloromethane.[4] The organic extract is dried with anhydrous sodium sulphate. The solid phase extraction of insecticides with microcolumns of charcoal and Tenax[19] and also with phases used for liquid chromatography[19–21] is extremely promising. The concentration factor may reach 1000 which permits the use of small volumes of water or eluates and excludes removal of solvent prior to gas chromatographic analysis (GC).

For soils an average test sample is first taken by quartering from the air-dried mixed specimen, roots and stones removed, and the sample then ground and screened through a sieve of pore diameter 0.5 mm. The efficiency of recovering insecticides from soils depends markedly on their type and their absorptive powers. Consequently it is suggested that the the optimum means of extraction of insecticides is selected for each test sample from three parallel subsamples.[7] After keeping the dry sample in distilled water the insecticide is extracted in the first extraction method with a mixture of hexane–acetone 4:1, in the second with a mixture of acetone–hexane 1:1 in the presence of 1% ammonium chloride solution and in the third with acetone with the same salt added.[7,17]

Preliminary deactivation of soils with ammonium chloride solution and subsequent extraction of insecticides in an ultrasonic bath with acetone and further re-extraction with hexane frequently enables analysis of extracts by GC with an electron capture detector (GC/ECD) without further purification.[22] It was shown that treatment of soils with 20% BF_3 in methanol prior to extraction increased the amount of HCCH isomers extracted by up to three times compared to any other extraction method.[23]

Insecticides are extracted from sludges, after homogenisation, with acetone[6] or with dichloromethane.[4]

Insecticides are often extracted from biological material with acetone–hexane mixtures in various proportions with simultaneous homogenisation of the biosample.[11,17] Acetonitrile or acetone is usually used for plant tissues.[24] Insecticides are then quantitatively transferred from water-soluble solvents to water-insoluble solvents, usually into hexane, cyclohexane or dichloromethane for purification.

Most frequently biosamples, after homogenisation, are dried with anhydrous sodium sulphate at a ratio of 1:4, the mixture is ground to a powder[5,10,14,25,26] and the insecticides extracted with an organic solvent in a chromatographic column or in a beaker.

Insecticides are usually extracted from milk with solvents,[16] however recently it has been suggested that extraction is carried out after adsorption of milk components on special cartridges,[27] silica gel[28] or Florisil.[29]

When it is important to know the lipid content of the biosample, extracts are evaporated to dryness, the mass of lipid determined in the dry residue, which is then dissolved in organic solvent for purification.

3. Purification of organic extracts

Removal of organic substances co-extracted with insecticides which interfere with analysis is achieved by treatment of hexane extracts with concentrated sulphuric acid until a colourless layer is obtained.[1–3,6,7,17,30] This method of purifying extracts is only used when acid-stable insecticides are being determined, for example DDT and its metabolites, HCCH isomers, hexachlorobenzene (HCB) and polychlorinated biphenyls (PCB). When analysing acid-unstable insecticides extracts are purified on adsorbents in chromatographic columns.

It should be mentioned that elemental sulphur and organosulphur compounds are often present in organic extracts of soils and sludges. These are not removed by acid treatment but interfere with the determination of HCCH isomers. Sulphur compounds are removed by adding to the hexane extracts solutions of tetrabutylammonium sulphate and sodium sulphate in the presence of isopropyl alcohol[6,7] or activated metallic copper.[4]

Insecticides of low stability in acid media, and they are the overwhelming majority, are separated from the interfering organic matrix usually on chromatographic columns of Florisil, silica gel or Al_2O_3,[5,8,11,13–16,18,21,24–27,29–32] or by high performance liquid chromatography (HPLC).[33] This purification may be automated.[34] A very efficient means of separating insecticides in extracts of animal and plant tissues is exclusion (gel-permeation) chromatography,[10,11,24,26,35] one of the variants of HPLC. Styrene-divinylbenzene gels, such as BioBeads SX2 or SX3, are used for this. Only 0.02% lipid remains in extracts after this treatment.[11]

4. Separation of insecticides in extracts

Extracts of insecticides (DDT, DDE, DDD and HCCH), purified from organic substances, may be analysed in gas chromatographs with packed columns only when contamination by PCB and many acids-unstable insecticides which interfere with the analysis is absent. In the majority of samples however, such contaminants are present and consequently extracts are usually fractionated.

Extracts of insecticides purified with sulphuric acid are subjected to alkaline dehydrochlorination with alcoholic alkali. In this case hydrochloric acid is split out from molecules of DDT, DDD, and HCCH while molecules of DDE, PCB and HCB are practically unchanged.[1,2,6,7,15] It should be mentioned that on Florisil, in addition to separating intefering organics from insecticides, it is possible to separate PCB from insecticides[13] and resolve insecticides into fractions[10,11] by changing the eluting solvents.

In the majority of cases extracts of insecticides purified on Florisil are resolved into fractions on silica gel,[5,8,26,30] after which they are analysed by gas chromatography.

The simultaneous purification and fractionation of insecticide extracts on one chromatographic column of aluminium oxide and silica gel[25,31] is of practical interest. This enables time and solvent volumes to be reduced.

5. Gas chromatographic separation and detection limits

Purified fractions of insecticides are analysed by GC/ECD usually on packed columns. If PCB are present in the extracts then fractions are analysed in the chromatograph prior to and after dehydrochlorination. The limit of detection of insecticides and PCB in sea and fresh water, depending on the sample volume taken, is equal to: for DDT and its metabolites 0.03 [3] and 0.2–3.8 ng litre^{-1},[1,2] α- β-, γ- and δ-HCCH 0.01 [3] and 0.2–1.3 ng litre^{-1},[1,2] PCB 0.2–20 ng litre^{-1};[1,2] in sludges: for DDT and its metabolites 0.3–0.5 [6] and 5 ng g^{-1} (SP-2250 or SP-2401 columns),[5] lindane and heptachlor 0.,1 [6] and 5 ng g^{-1},[5] α- and γ-HCCH, aldrin etc. (13 insecticides in all) 5 ng g^{-1},[5] PCB 30 [7] and 20 ng g^{-1};[5] in fish: for DDT and its metabolites 0.6 μg g^{-1} of fish fat,[17] 21 insecticides 10 ng g^{-1},[26] 19 insecticides 2–40 ng g^{-1},[10] PCB 0.6 μg g^{-1} fish fat[17] and 100 ng g^{-1};[26] in soils: for DDT and its metabolities 2–3 ng g^{-1}, α- and γ-HCCH 0.2 and PCB 30 ng g^{-1} dry weight.[7] Retention times for 28 insecticides on 9 different packed columns have been published.[36]

Recently insecticides and PCB have also been analysed on quartz capillary columns. This enables the simultaneous determination of significantly more insecticides from only two fractions.[11,26,31] Thus it is possible to determine 19 insecticides and 7 PCB isomers in biosamples on columns of length 25 m of CP-Sil 5 CB or CP-Sil 8 CB,[31] 37 insecticides in human adipose tissue on columns of length 15 m of DB-5 or DB-17 with a limit of detection of 0.9–14.3 ng g^{-1},[11] 28 insecticides in topsoil on columns of length 25 m of OV-1701, OV-17 and SE-52 [22] and 12 insecticides in water

on a column of length 30 m of SPB-608 with a limit of detection 10 ng litre^{-1} [by GC-mass spectrometry (GC-MS)].[19]

III. Biphenyls

1. Sample treatment

The PCB are a mixture of substances used as oils for transfomers, hydraulic fluids, dielectrics, etc. In different countries PCB have their own trade names, for example trichlorobiphenyls in the USSR, Sovols in France, Arochlors in USA and Chlophens in FRG. They are in turn divided into types reflecting the percentage chlorine content in the mixture and the number of chlorine atoms in the molecule. For example, Arochlor 1254 series 1200 contains 54% chlorine, it has an average of 4.9 chlorine atoms per molecule and an average molecular weight of 324, Chlophen A-50 contains 50% chlorine, and a pentachlorobiphenyl of the type of the electrochemical Sovol has 54%.[7] These are frequently used as standards in the determination of PCB.

Since PCB are met, as a rule, in the environment together with the various chlorine-containing insecticides, all these substances are usually determined in one sample. PCB are separated from acid-stable insecticides by alkaline dehydrochlorination and chromatograms are compared prior to and after dehydrochlorination. Insecticides are determined from the difference in peaks on the two chromatograms and PCB from the total peak heights in the second chromatogram. PCB are usually separated on silica gel (see above) when determining acid-stable insecticides. The limit of detection of total PCB content in natural water is 0.2–20 ng litre^{-1},[1,2] and in sea sludges and soils 2–5 ng g^{-1} dry weight respectively.[6,7] Chlophen A-50 is used as a standard for PCB.

When determining PCB the selection, storage and treatment of samples of environmental objects is generally the same as for insecticides (section II). Mention may also be made of the concentrating of PCB from contaminated natural waters with adsorbent C_{18},[37] the purification of extracts by HPLC with subsequent determination of PCB by GC/MS,[38] loss of the overall PCB content up to 56% and of each isomer separately when drying sludges at 60 °C for 48 h,[39] and also the possibility of combining purification and determination in one sequential system LC-GC/ECD,[40] which enables determination of PCB to be automated.

Methods have been described for determining many individual PCB isomers in various biological samples, such as whales,[41] penguins and their eggs,[42] molluscs,[43] sea mammals,[44] fish,[45–50] animal tissues,[51] milk [52] and human blood.[53] It is necessary to mention that PCB isomers from bound fats (phospholipids) must be separated quantitatively by saponification with subsequent extraction with polar solvents.[39]

2. Gas chromatographic separation and determination

The quantitative determination of PCB for individual types of PCB, such as Chlopen A-50 or Arochlor 1254, is difficult since different types of PCB or mixtures may be present in the environmental samples. Consequently several individual types of PCB or mixtures have come to be used as standards in recent years. For example, Arochlors 1242, 1248, 1254 and 1260 are used as standards for the determination of PCB in soil and grass with a limit of detection of 1 ng g^{-1} dry weight.[54] The same standards are used for lake deposits but with quartz columns,[55] and a mixed standard containing equal amounts of Arochlors 1242, 1254 and 1260 is used for soil with a limit of detection of 0.5 ng g^{-1}.[56]

Cross-calibration in the determination of actual amounts of Arochlors 1242 and 1254 and their sum in contaminated residues using seven individual standards containing Arochlors 1221, 1232, 1016, 1242, 1248, 1254 and 1260 showed that the relative standard error of the GC/ECD method using packed columns was 30% and of the GC/MS method using capillary columns was 38%.[57] Occasionally internal PCB standards, such as 2,4,5-trichloro- and 2,2',3,4,5,6-hexachlorobiphenyl are added to test samples of sludges and entrapped water with subsequent determination by GC/ECD on capillary columns with 84–99% detection of standards.[58]

In view of the importance of Arochlors as standards the percentage of isomeric groups is determined in each of them by GC/MS. For example, Arochlor 1254 has Cl_4 16.0, Cl_5 59.9, Cl_6 23.8 and Cl_7 0.4% and Arochlor 1268 has Cl_7 4.0, Cl_8 36.3, Cl_9 52.3 and Cl_{10} 7.4%,[59] and a series of Arochlors may contain up to 60 individual isomers.[60]

The inadequacy of comparing concentrations in a test sample with that of determined Arochlor or Chlophen is reinforced by the change with time of the 'PCB picture' in the environment as a result, for example, of isomerically specific disintegration, biotransformation and bioaccumulation by living organisms. Furthermore the problem of quantitatively determining PCB will be aggravated by the removal by nature of individual PCB isomers in chemical and pyrolytic processes. In addition to the reasons mentioned above the precision of determination in environmental samples will also markedly depend on the variability and sensitivity of each isomer to the method being used.

In all 209 PCB isomers are known: Cl_1 (3), Cl_2 (12), Cl_3 (24), Cl_4 (42), Cl_5 (46), Cl_6 (42), Cl_7 (24), Cl_8 (12), Cl_9 (13), Cl_{10} (1), each has been given a number from 1 to 209.[60–62]

For more precise determination of PCB in samples the use of calibration mixtures of PCB isomers at various concentrations has been proposed. These include one isomer from each isomeric group, with a number of chlorine atoms from 1 to 10, and analysis by GC/MS.[63] Identification and quantitative determination of PCB isomers by the actual number of chlorine atoms is effected by their detection in a particular mass range in the mass spectrogram. For example all the Cl_4 isomers are in the mass range 288–294 and all the Cl_7 in 390–396. Using the same principle a further 21 polychlorinated insecticides may successfully be determined in one test sample in addition to PCB.[64] With the same set of standards it is possible to determine PCB in soils by GC/MS with positive chemical ionisation.[65]

For the precise determination of the total PCB content in a sample it is necessary to determine all the PCB isomers present in it. It is necessary that each isomer appears on the chromatogram as a single peak well separated from other PCB isomers or compounds. This makes it possible to avoid dubious results from the variable contribution of co-eluting isomers in an unresolved peak and also from differences in sensitivity coefficients in the ECD. Consequently the precise determination of the total PCB content in test samples must be carried out with a set of all the individual PCB isomers as standards and not with mixtures. This is impossible in practice although all 209 PCB isomers have recently been synthesised and their retention times determined.[66]

Determination of the total content of PCB or of separate isomers by high resolution gas chromatography (HRGC)/ECD is carried out at present on capillary columns of SE-54 phase (DB-5, CP Sil 8, HP-5) of length 25–60 m. However all isomers encountered in

natural test samples cannot be completely separated on just one column. Nonetheless the relative elution order of PCB isomers has been determined.[62,66] With a fixed substitution pattern in the first ring the retention of this family of isomers increases in the orders: 2 < 3 < 4; 2,6 < 2,5 < 2,4 < 2,3 < 3,5 < 3,4; 2,4,6 < 2,3,6 < 2,4,5 < 2,3,4 < 3,4,5; 2,3,5,6 < 2,3,4,6 < 2,3,4,5. The relative elution order of the PCB isomers remains the same for polar phases such as OV 1701 etc.[62] It should be mentioned that the separation of PCB isomers on columns with phases SE-30, SE-54 and OV-101 differ a little depending on the column manufacturer. This is linked with different methods of surface deactivation prior to coating with a film and immobilising the film itself.

Complete separation of all PCB isomers in complex mixtures was successfully achieved only in 1988 using two-dimensional GC [67,68] carried out on a Sichromat-2 (Siemens), fitted with two independent ovens and two ECD (main and monitor). A chromatogram of the mixture of PCB isomers being analysed is obtained with the first column (SE-54, 25 m × 0.32 mm) in the first oven. The main portion of the eluate, corresponding to the part of the chromatogram with complete separation of isomers, is then passed through the monitor detector and selected fractions of the eluate, corresponding to the unresolved isomers on the chromatogram, are directed to the second column (OV-210, 30 m × 0.32 mm) in the second oven with the main detector. The monitor detector on the first column shows the usual gas chromatogram of a mixture by ECD except for one or several breaks, corresponding to the eluate fractions directed to the second column. The main detector on the second column shows chromatograms of the separated isomers in the fractions of the eluate directed from the first column. The principle of the method and the technical details have been described previously.[69]

The chromatographic separation of isomers on the second column may be very efficient. For example, the cluster of three isomers Nos. 126 (3,3',4,4',5-), 129 (2,2',3,3',5-) and 178 (2,2',3,3',5',6-) have retention times on the first column of 26.81, 26.80 and 26.85 min respectively and on the second of 37.98, 37.13 and 36.48 min, i.e. the unresolved triplet was separated into three separate peaks.[67]

Extremely important problems are the criteria for selection of standard PCB isomers for analysis as a function of the range of chlorine numbers (the number of chlorine atoms in the PCB isomers) and their contribution to environmental test samples, the use for calculation of the total PCB content, the relative distribution between various phases of natural aqueous media such as the atmosphere, water, sludges and biological specimens, aqueous and lipid solvents, the tendency to bind to particles and lipids, persistency and finally toxicity.

The first criterion, considering only a number of isomers covering a wide range of physicochemical properties, may be used for specific purposes such as a comparison between types of sample and regions and also for directing analyses. However choosing isomers, based on a range of chlorine numbers together with a requirement for their significant content in mixtures of the Arochlor type, for precise determination leads to a more or less arbitrary selection of isomers, at least in an ecological sense. The limitation of data by isomer is probably not the most correct approach for an answer to many problems linked with the behaviour and effects of PCB in the environment. Examples are the set of the ICES [?International Commission for the Exploration of the Seas (Ed. of Translation)] consisting of six isomers with chlorine numbers 3–7: No. 28 (2,4,4'-), 52 (2,2',5,5'-), 101 (2,2,',4,5,5'-), 153 (2,2',4,4',5,5'-), 138 (2,2',3,4,4',5-) and 180 (2,2,',3,4,4',5,5'),[68] a mixture of Arochlors 1221, 1016, 1254, 1262 (20:10:7:6) for the analysis of environmental samples,[70] a set of fourteen isomers: Nos. 28, 52, 74 (2,4,4',5-), 66 (2,3',4,4'-), 101, 151 (2,2',3,5,5',6-), 118 (2,3',4,4',5-), 153, 105 (2,3,3',4,4'-), 138, 187 (2,2',3,4',5,5',6-), 183 (2,2',3,4,4',5',6-), 156 (2,3,3',4,4',5-) and 180, comprising about 70% total PCB content in human milk.[71,72]

The set of PCB isomers based on their toxicity is important. Quantitative structure–activity relationships show that the most toxic PCB isomers are isostereomeric with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.[51,73] It is proposed that their toxicity is explained by the presence of chlorine atoms in positions 4,4' and/or 3,3,',5,5' and also by the possibility of two benzene rings achieving a planar configuration when one or both *ortho* positions are not substituted by chlorine atoms. This condition is satisfied by 13 isomers: Nos. 37 (3,4,4'-), 77 (3,3',4,4'-), 81 (3,4,4',5-), 118, 123 (2',3,4,4',5,5'-), 114 (2,3,4,4',5-), 105, 126 (3,3',4,4',5-), 167 (2,3',4,4',5,5'-), 156, 157 (2,3,3',4,4',5-), 169 (3,3',4,4',5,5'-) and 189 (2,3,3',4,4',5,5'-). It has been proposed that the toxicity of PCB isomers be considered the most important criterion for determining them in evironmental samples. Only four toxic isomers (Nos. 81, 114, 169 and 189) may be determined on one column of phase SE-54 and the remaining isomers may only be determined by two-dimensional GC.[68]

Mention must be made of the influence of the quality of appropriate PCB standards used on the precision of determining PCB isomers. PCB isomers of inadequate or inapplicable purity are available for sale. This leads to the development of additional peaks in the spectrum when working with an ECD or flame-ionisation detector (FID) and to the splitting of peaks on various columns. Consequently the use of calibration standards of mixed PCB solutions consisting of such individually inadequately pure PCB isomer standards may lead to large qualitative and quantitative errors when determining separate isomers and overall PCB content.[68]

IV. Herbicides

Chlorine-containing herbicides are widely used in agriculture for the elimination of weeds and undesirable woody undergrowth and as defoliants. The most important of them are acidic in character and consequently they are determined, as a rule, as esters by GC/ECD. Their determination by HPLC has only been described in the form of free acids in the concentration range 20 ng litre^{-1} to 1 mg litre^{-1}.[74]

Usually test samples of water[74–77] or sludge[77] are acidified to pH < 2 and the herbicide extracted with organic solvent, then esterified and the ester purified on columns of silica gel or Florisil.[75–77] Diazomethane[75] and pentafluorobenzyl bromide[76,77] are used as derivatising agents. The limits of detection of the methods are 0.05 μg litre^{-1},[76] 0.5 μg litre^{-1},[75] and 10–25 μg kg^{-1}.[77] Determination is carried out on packed[75,77] and capillary columns. It was shown that the choice between pentafluorobenzyl and methyl esters for the determination of herbicides depends on the relative importance of the detection limits and the linear range of concentration.[78]

The determination of 18 (cyanoethyl)dimethylsilyl derivatives of herbicides by the GC/N–P detector made it possible to exclude purification of the sample, which had been required for GC/ECD, and to have a limit of detection of 0.1 μg litre^{-1}.[79] The same method has been proposed for the determination of herbicides in solid discharges.[80] Capillary columns of length 30 m with phases DB-5 [76,78,80] and SP-2250 [78] and of length 15 m with phase DB-1 [79] have been used. The retention times, linear range of concentration

and sensitivity coefficients for herbicide esters have been determined for the separate columns.

V. Phenols

Polychlorophenols are some of the main contaminating substances in the environment, particularly pentachlorophenol which is the most widespread wood preserving agent, and is also a disinfectant and pesticide. 2,4-Dichloro- and 2,4,5-trichlorophenols are formed on breakdown of the widespread herbicides 2,4-D and 2,4,5-T and accumulate in nature. Trace quantities of polychlorophenols are determined as a rule in environmental objects as esters which are freed more easily from the interfering influence of other compounds and are separated on chromatographic columns.

Polychlorophenols are usually extracted from test samples of natural waters with organic solvents and are acetylated at the same time with acetic anhydride.[74-84] The same procedure is applied to sludges,[82] or polychlorophenols are first extracted and then acetylated.[85,86] The degree of extraction of chlorophenols from residues, however, depends markedly on the method used and consequently serious difficulties are encountered in the interpretation of the results obtained. This was shown when comparing methods of isolating chloroguaiacols and chlorocatechols from contaminated test samples.[87] From biosamples (homogenised tissue) chlorophenols are separated by a mixture of solvents with anydrous sodium sulphate[88] or dilute sulphuric acid.[89]

Chlorophenoxyacetates isolated from natural waters are not purified before determination but from sludges and particularly from biosamples they are usually separated from interfering organics on silica gel for example.[86,88] Test samples of chlorophenols are stored under the same conditions before analysis as when determining organochlorine pesticides.

Chlorophenoxyacetates are determined by GC/ECD on capillary columns of length 12-30 m with phases OV-73,[81,82] OV-1701 and SE-54,[85,88] OV-1 [86] and DB-1,[84] and by GC/MS with phases DB-1 [84] and SPB-5.[86] The limits of detection are 1 ng litre^{-1} for natural waters[81] and 0.2 ng litre^{-1} for sludges.[86] The limit of detection for chloroanisoles and -veratroles from a wet mass of fish tissue is in the range 0.2-10 ng g^{-1}.[88]

It was shown that the determination of chlorophenols in water as acetates has significant advantages compared with determination as pentachlorobenzyl ethers because the acetylation can be carried out directly in the test sample, reducing matrix influences and improving detection. By GC/MS it is possible to determine up to 35 haloesters from one test sample, including phenol and monochlorophenols which are usually difficult to determine by GC/ECD.[84]

In recent years the chromatographic and mass spectrometric properties of upwards of 30 chlorophenols and various esters have been studied in detail. Absolute limits of detection, relative retention times and sensitivity coefficients have been determined for chlorophenols (GC/ECD),[90] (GC/FID),[91] acetates (GC/ECD),[90] (GC/ECD, GC/MS),[84] anisoles,[90] pentafluorobenzyl ethers (GC/ECD),[91-93] (GC/ECD, GC/MS) [84] (cyanoethyl)dimethylsilyl ethers (GC/N-P-detector, GC/MS).[94] These data will permit the improvement and simplification of effective methods of determining chlorophenols in environmental objects in the near future.

Chlorophenols are rarely determined by other methods without derivatisation. They are usually separated from water and concentrated on solid adsorbents, such as polydivinylstyrene,[95] graphite [96] and XAD resins,[97] and then determined by HPLC on columns with C_{18} phases [95,96] or GC/IR spectroscopy.[97] Limits of detection are 4-40 ng litre^{-1}.[96]

VI. Dioxins and furans

Polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) are tricyclic aromatic compounds with similar physical, chemical and biological properties. They are the reason for the mass illness and poisoning of people in Seveso (Italy) in 1976, Japan and USA in 1971-1983, and particularly at the time of the war in South Vietnam in 1968-1973.

The number of chlorine atoms in these compounds varies from 1 to 8 and the total number of positional isomers in PCDD and PCDF is 75 and 135 respectively (Table 1), they have been numbered systematically with indices D and F. For example the most toxic isomers 2,3,7,8-TCDD and 2,3,7,8-TCDF have the symbols D 48 and F 83 respectively.[98] All these isomers differ significantly in acute toxicity and biological activity (up to 300 000 fold). The most toxic and bioactive are 12 isomers with 4-6 chlorine atoms, of which 4 must be in positions, 2,3,7 and 8 (Table 2). Several of these isomers have oral LD_{50} levels < 10 μg kg^{-1} for guinea pigs.[99]

Table 1. Number of positional isomers of PCDD and PCDF.

Level of chlorine substitution	Number of isomers	
	PCDD	PCDF
Cl (mono)	2	4
Cl_2 (di)	10	16
Cl_3 (tri)	14	28
Cl_4 (tetra)	22	38
Cl_5 (penta)	14	28
Cl_6 (hexa)	10	16
Cl_7 (hepta)	2	4
Cl_8 (octa)	1	1
Total	75	135

Table 2. The most toxic PCDD and PCDF isomers.

PCDD	PCDF
2,3,7,8-Tetra-CDD	2,3,7,8-Tetra-CDF
1,2,3,7,8-Penta-CDD	1,2,3,7,8-Penta-CDF
1,2,3,6,7,8-Hexa-CDD	2,3,4,7,8-Hexa-CDF
1,2,3,7,8,9-Hexa-CDD	1,2,3,6,7,8-Hexa-CDF
1,2,3,4,7,8-Hexa-CDD	1,2,3,7,8,9-Hexa-CDF
	1,2,3,4,7,8-Hexa-CDF
	2,3,4,6,7,8-Hexa-CDF

PCDD and PCDF are found in trace concentrations in industrial chemical products such as polychlorophenols and their derivatives, and herbicides based on phenoxyacids and diphenyl ethers.

They are found in combustion products of industrial and communal incineration furnaces, on pyrolysis of PCB, chlorophenols, chlorobenzenes and many other chlorine-containing organic compounds.[100,101]

Modern methods for the quantitative determination of the isomers of PCDD and PCDF in environmental objects at concentrations at the ppt level will be considered in this chapter. Principal attention will be paid to the most toxic isomer, viz. 2,3,7,8-TCDD. Some of this material has been correlated in reviews.[100-102]

In view of the extremely low hazardous concentrations of PCDD and PCDF the quantitative content of their separate isomers may only be determined by HRGC and MS, since the former is capable of separating all isomers but is insufficiently selective with a background of matrix interference and the second identifies isomers and is extremely sensitive in the whole mass range at a level of 10^{-13} g on using high or low resolution mass spectrometry. However without HRGC, mass spectrometry does not resolve the majority of isomers because many of them give very similar mass spectra.[101,102]

1. Selection and storage of samples

Test samples of air containing PCDD and PCDF are taken in equipment consisting of sequentially connected filters for the removal of aerosols and cartridges of various adsorbents for trapping gaseous products. Glass fibre,[103-105] quartz wadding,[106] and as adsorbents XAD-2 resin,[105,107] polyurethane foam,[103,108] silica gel [104] and ethyleneglycol [106] are used as filters. A 6-1000 m^3 volume of air is passed through the filters and adsorbents under vacuum at a rate of 1-14 $m^3\ h^{-1}$.

In order to concentrate PCDD and PCDF from drinking water it has been proposed to pass the latter (10-200 litre) through a cartridge of XAD-2 resin [109] or C_{18} phase [110] at a rate of 0.15 to 0.5 litre min^{-1}.

Soil samples up to 3 cm from the surface layer and deeper layers are taken with tubular samplers and stored before analysis in wide mouthed glass jars with teflon screw caps at 4 °C [111] or −30 °C.[112]

After taking and before analysis biosamples must be rapidly frozen if possible and stored in the frozen state down to −180 °C, as a rule, in aluminium foil. This was shown for fish,[112-115] seals,[116] rabbits and pheasants,[112] human tissue [117-119] and vegetable fruit.[112] Temporary storage in the cold state is possible in glass vessels for test samples of cows' cream and earthworms (after washing and homogenisation).[112]

2. Preparation of samples for analysis and extraction

Samples need to be carefully homogenised and often dried for the quantitative recovery of PCDD and PCDF isomers from environmental samples using organic solvents.

Test samples of soil and sludges are dried in air or in a desiccator to constant weight at 60-110 °C and moisture content is usually determined. They are then screened through a sieve to remove foreign bodies (stones, shells, roots, etc.).[111,120-122] Test samples are also dried by mixing with anhydrous sodium sulphate [123-125] or by lyophilisation.[126]

Biological test samples are homogenised by alkaline,[112,113,118,127-129] acidic [115,129-131] or enzymic [132] degradation, lyophilisation and subsequent crushing,[129,133] grinding in a meat grinder or homogeniser with subsequent powdering with anhydrous sodium sulphate.[114,117,123,129,134-138]

For the complete identification and precise quantitative determination of PCDD and PCDF isomers it is necessary to introduce precise amounts of a mixture of isotopically labelled PCDD and PCDF standards. These are added to the homogenised test sample (biological tissue, soil, sludges, etc.) and to samples taken from various filters and to cartridges (from air and various types of water) before extracting them with organic solvent. The standards have a definite number of chlorine atoms including tetra- (T), penta- (Pe), hexa-, hepta-, and octa- (O)-CDD and -CDF, 10 isomers in all (0.1-3 ng of each). All the standards in Table 2 are used for the quantitative determination of all the most toxic isomers. The isotopically labelled standards $^{13}C_{12}$-PCDD/PCDF and less frequently $^{37}Cl_{4-8}$-PCDD/PCDF are generally used.[100-102,139] It should be mentioned however that these standards are very expensive. Consequently the whole set are used in only a few laboratories in the world. The majority use only a few standards which positively reduces the precision of determining and identifying PCDD/PCDF.

In addition to using filters for aerosols and cartridges for air, PCDD and PCDF are extracted from samples in a Soxhlet apparatus usually with toluene,[103-105,108] and from soils and sludges with various organic solvents or solvent mixtures.[111,121,122,124,125,140,141] It should be mentioned that the degree of recovery of PCDD/PCDF is increased by previous treatment of samples of soil, sludges and aerosols with hydrochloric acid prior to extraction or carrying out extraction combined with ultrasonic treatment.[142]

PCDD/PCDF are extracted from water with dichloromethane[125,141] and, after concentrating them on a cartridge with adsorbents, with acetonitrile or mixtures containing it.[109,110] PCDD/PCDF are extracted from homogenised biological samples such as fish tissue,[112-115,121,123-129,131,133,135,138] adipose tissue,[116-118,123,134,137,143,144] blood plasma,[119,144] milk [130,132,136,145] or human tissue [139] most frequently of all with hexane or mixtures of it with other organic solvents.

3. Purification of organic extracts

In addition to PCDD and PCDF, many other co-extracted substances and compounds of biogenic (lipids, proteins, etc.) and anthropogenic occurrence are contained in organic extracts of samples from environmental objects, which it is necessary to separate before determining dioxins and furans by GC/MS.

A multistage and very laborious purification of extracts is required when determining PCDD and PCDF by low resolution GC/MS (on packed columns). For example, extracts of human milk are purified on four chromatographic columns containing as adsorbents: 1) silica gel/alkali on silica gel/acid on silica gel; 2) silica gel/acid on silica gel; 3) silver nitrate on silica gel; 4) aluminium oxide and two columns of reversed and normal phase HPLC.[130]

The requirement for purification of organic extracts is reduced with the introduction of HRGC on capillary glass and particularly quartz columns with bonded phases and of length 30-60 m. It should be mentioned that purification methods vary strongly in relation to matrix complexity and in the case of biological samples it is necessary to purify extracts from far larger quantities of interfering substances and compounds than in the case, for example, of samples of atmospheric air.

After purification all the PCDD/PCDF isomers are concentrated as a rule on a column of carbon of type PX-21 (Amoco) or Carbopak (Supelco) from which they are eluted usually with toluene for analysis by GC/MS.

Extracts of air samples require simpler methods of purification. Their purification has been proposed on two columns of modified silica gel/silica gel and carbon on celite 545 [105] or aluminium oxide,[108] on a column of Florisil and normal phase HPLC,[103] on three columns with basic and acidic aluminium oxide and with activated carbon.[104]

Extracts of water, sludge and particularly biological samples, are usually subjected to complex and careful purification for determining the large number of individual isomers of various groups of PCDD and PCDF. Often however only 2,3,7,8-TCDD or other individual isomers or groups of isomers are determined. In this event the purification of extracts is significantly simplified. It must also be emphasised that it it possible to determine all PCDD and PCDF isomers from one fraction when purifiying extracts by adsorption column chromatography.[123] When using purification by normal or reversed phase HPLC in the last stage of purification several fractions must be used. Consequently these methods are usually used for determining separate isomers or groups of isomers.

Organic extracts of water are purified with conc. H_2SO_4 prior to decolourisation and on columns of Florisil and carbon/celite 545,[109] on columns of silica gel/acid on silica gel, of acid aluminium oxide and carbon/celite 545.[125]

Extracts of sludges are purified on columns of carbon/celite 545 and of silica gel with subsequent normal and reversed phase HPLC,[122] after removal of sulphur on columns of acid on silica gel, alkali on silica gel, $AgNO_3$ on silica gel, basic Al_2O_3, reversed phase HPLC,[126] exclusion chromatography, sequential treatment with Na_3PO_4, H_2SO_4, on columns of Al_2O_3 and carbon,[131] on columns of silica gel/Na_2CO_3/alkali on silica gel/H_2SO_4 with $CaSO_4$/silica gel/alkali on silica gel, acid Al_2O_3, carbon/celite 545 and neutral Al_2O_3.[121] Soils were purified as for water,[125] on columns of CsOH/silica gel/acid on silica gel and on Al_2O_3,[111] contaminated soil on columns of CsOH/silica gel/acid on silica gel and $AgNO_3$/silica gel.[111]

It has been proposed to purify extracts of fish tissue on columns of MgO/celite 545/Al_2O_3 and Florisil/Al_2O_3,[133] on a column of acidic Al_2O_3/basic Al_2O_3 and then by normal and reversed phase HPLC,[128] on three columns of H_2SO_4/silica gel, NaOH/silica gel or $AgNO_3$, basic Al_2O_3 and reversed phase HPLC,[115] by exclusion chromatography and reversed phase HPLC on two different columns,[127] by exclusion chromatography and on columns of Al_2O_3 and Florisil,[138] on four columns with H_2SO_4 and NaOH with silica gel and by HPLC,[112] on three columns of celite 545/conc. H_2SO_4, Cs_2SiO_3 and carbon,[114] and on columns of silica gel/K_2SiO_3/H_2SO_4/celite 545, Al_2O_3, carbon/silica gel [135] as in the case of sludges.[121,131]

Extracts of human tissue samples are purified by treatment with H_2SO_4, on columns of Florisil and carbon/silica gel,[139] adipose tissue on three columns of silica gel/alkali on silica gel/acid on silica gel, $AgNO_3$/silica gel, basic Al_2O_3 and then normal and reverse phase HPLC,[146] on three columns of acid on silica gel, Al_2O_3, carbon/celite,[117] by exclusion chromatography on columns of Florisil and of carbon,[143] by exclusion chromatography on columns of Florisil, Al_2O_3 and carbon/celite 545,[116] by treatment with H_2SO_4, on columns of silica gel, carbon/silica gel, by GC,[118] human blood plasma on columns of acid on silica gel/silica gel and carbon/celite 545,[144] as for adipose tissue,[119] and human milk by a modified procedure [136] for adipose tissue.[146]

Among the contemporary methods of preparing samples and analysing by GC/MS a procedure has been evolved combining the extraction and purification of samples of soil, sludges and biological specimens into a two stage process and enabling determination of all the PCDD and PCDF isomers from one fraction.[123] In the first stage the mixture of homogenised test sample and Na_2SO_4 is extracted in the first column and the extract passed in the same column through K_2SiO_3/silica gel, and then through two columns of Cs_2SiO_3 or K_2SiO_3/silica gel and activated carbon. With the exception of *ortho*-PCB, the PCDD, PCDF, polychlorinated naphthalenes (PCN), biphenyls (PCBP) and a series of polyaromatic hydrocarbons remain on the carbon adsorbent. In the second stage the extract is passed through two columns of Cs_2SiO_3/acid on silica gel and aluminium oxide. Modifications of this procedure have been applied successfully to the determination of PCDD/PCDF isomers in human adipose tissue,[134,144] and also in human and cows' milk.[145,147]

It is evident from the material presented above that the purification of extracts for subsequent determination of PCDD and PCDF by GC/MS is a complex and prolonged process in the majority of cases. The main part of the time is consumed in this way, occasionally reaching 80% (10 h or more) of the time for carrying out the whole analysis. Consequently attempts have been made to reduce the time of purification of extracts for which a modified method of separation and identification by GC/MS/MS has been proposed.[109,148–150]

The method of tandem mass spectrometry is based on the separation of the components of a mixture according to their molecular weight in the first analyser of the mass spectrometer (low or high resolution) and obtaining structural information on the determined compounds in the second analyser at low resolution. It is impossible to use these separating capabilities of the MS/MS method for the direct analysis of extracts in view of the extremely low concentrations of PCDD and PCDF in comparison with the main interfering compounds. Consequently it is necessary first to remove the latter from the compounds to be determined on short capillary gas chromatographic columns of length 12 m. This method has been used successfully for determining PCDD and PCDF in drinking water and soil and has enabled the time required for purification of extracts of biological samples to be reduced significantly by shortening several stages.

4. Gas chromatographic separation of isomers

Purified extracts of PCDD and PCDF are most frequently analysed by gas chromatography-mass spectrometry with quartz and, far more rarely, with glass capillary columns, usually 50–60 m long with a strongly polar (3-cyanopropyl)polysiloxane phase, such as SP-2330,[135,138,145] SP-2331,[105,126,136,144] CP-Sil 88 [118,125] and Silar 10C,[151] and with non-polar phase DB-5.[116,124,143,145–147] Columns of length 25–30 m with DB-5 have also been used.[116,135,137,139] The columns lead directly into the ion source of the mass spectrometer operating in electron impact (EI) or negative chemical ionisation (NCI) modes.

It must be emphasised however that at the present time a single column does not exist in which it would be possible to separate all the 2,3,7,8-substituted PCDD/PCDF isomers in the presence of all the PCDD/PCDF isomers. It is possible to separate all the 2,3,7,8-substituted isomers of PCDD successfully on columns of SP-2330 [151] and Silar 10C,[152] but in the case of the 2,3,7,8-substituted isomers of PCDF this proved to be impossible even within the TCDF group.[153,154] In the latter case complete separation was achieved only when using two columns such as SP-2331 and DB-17,[155] or on a column of a new smectic liquid crystal phase.[156]

The use of sequential combined columns of DB-5 and CP-Sil 88 of length 50–60 m must be mentioned. The various chlorine groups of PCDD/PCDF with equal numbers of chlorine atoms are separated on the first column and the isomers in these groups in the second. Such a method enables large volumes of solvent, up to 10 μlitre, to be injected into the gas chromatograph and significantly reduces the level of detection.[137]

Gas chromatographic characteristics have been described for all TCDD-OCDD for a Silar 10C column,[152] all PCDF for a DB-5 column,[157] for 35 PCDF for SP-2330 and V-101 columns,[158] for all TCDF for DB-5 and SP-2331 [154] and SE-54 and SP-2330 columns,[153] all TCDD for SP-2330 and OV-1 columns[159] and for columns with modified (cyanopropyl)polysiloxane phase,[160] and for the toxic PCDD/PCDF for columns with the new OV-240-OH phase.[161]

5. Mass spectrometric determination

The mass spectrometry of PCDD and PCDF has been described in detail in reviews[100–102,151,162,163] and will be considered here only briefly and as applied to the quantitative determination of isomers of xenobiotics in the environment.

Table 3. Ions (m/z) used for monitoring PCDD and PCDF in test samples of environmental objects.[101]

	Ions for monitoring	Tetra-	Penta-	Hexa-	Hepta-	Octa-
	PCDD					
EI	M^+	320	354	388	422	—
	$(M+2)^+$	322	356	390	424	458
	$(M+4)^+$	—	—	—	—	460
NCI	M^-	(320)	354	—	—	—
	$(M+2)^-$	(322)	356	—	—	—
	$(M-Cl)^-$	—	—	353	387	—
	$(M+2-Cl)^-$	—	—	355	389	423
	$(M+4-Cl)^-$	—	—	—	—	425
	PCDF					
EI	M^+	304	338	372	406	—
	$(M+2)^{+a}$	306	340	374	408	442
	$(M+4)^{+a}$	—	—	—	—	444
NCI	M^-	304	338	372	406	—
	$(M+2)^{-a}$	306	340	374	408	442
	$(M+4)^{-a}$	—	—	—	—	444

[a]PCN and PCT intefere a little, but interference from PCDPE is possible.

Low resolution quadrupole mass spectrometry is most frequently used for monitoring PCDD and PCDF. EI in the selective ion detection (SID) mode is used for the determination of TCDD/TCDF for which it has a high sensitivity (1–10 pg). However the sensitivity falls significantly for the more chlorinated isomers. On the other hand NCI in the SID mode is 1–2 orders of magnitude (10–100 fg) more sensitive, when using methane as carrier gas, for all the PeCDD-OCDD and TCDF-OCDF in comparison with EI but has a low sensitivity for all TCDD, only 20% of that for EI.

PCDD/PCDF ions used for monitoring in EI are M^+ (for tetra-, penta- and hexa-) and $(M+2)^+$ for hepta- and octachloro compounds, in NCI M^- for the less chlorinated isomers of PCDD and M^- and $(M+2-Cl)^-$ for the more chlorinated isomers. For PCDF M^- or $(M+2)^-$ (Table 3) are used.[101]

The use of high resolution mass spectrometry (5000–10 000) gives additional selectivity and the senstivity on EI in the SID mode may reach 10–200 fg for all TCDD(F)-OCDD(F). The use of high resolution mass spectrometry and of MS/MS for determining PCDD/PCDF isomers has been described in detail in a review.[102]

The quantitative determination of PCDD/PCDF isomers is based on measurements of peak area or height and comparison of them with calibration graphs of external standards and isotopically labelled internal standards. Introduction of the latter into test samples before extraction of PCDD/PCDF helps to explain their loss in the extraction process and in the purification of extracts. It also aids identification of isomers and their quantitative determination.

Table 4. Molecular ions of PCDD and PCDF.[101]

Molecular ions (m/z, M^+, M^-) (chlorination)	Compounds					
	PCDD	$^{13}C_{12}$-PCDD	^{37}Cl-PCDD	PCDF	$^{13}C_{12}$-PCDF	^{37}Cl-PCDF
Mono-	218	230	220	202	214	204
Di-	252	264	256	236	248	240
Tri-	286	298	292	270	282	276
Tetra-	320	332	328	304	316	312
Penta-	354	366	364	338	350	348
Hexa-	388	400	400	372	384	384
Hepta-	422	434	436	406	418	420
Octa-	456	468	472	440	452	456

The following are necessary[101] for proof of identity of PCDD/PCDF isomers. The retention time of the PCDD and PCDF isomers being determined must be within 3 sec to establish the isomeric specificity and must be confirmed daily. The signal to noise ratio must be at least 2.5:1. The chlorine cluster must be ±10% of the theoretical value and precise fragments, such as $(M-COCl)^+$ ions, must coincide with the precise chlorine cluster.

In spite of the use of fairly effective methods of purification of extracts the presence is occasionally encountered of other contaminating polychlorinated compounds interfering with analysis. These may be mainly non-*ortho*-substituted PCB, PCN, PCBF, polychlorinated terphenyls (PCT), diphenyl ethers (PCDPE), pyrenes (PCP) (Tables 4 and 5) and also occasionally polychlorinated anthracenes, phenanthrenes and fluorenes. These compounds however are usually readily distinguished by their intense M^+ or M^- ion and the number of chlorine atoms deduced from their ionic clusters. In rare cases polychlorinated benzenes, benzofurans and styrenes are observed, but they do not interfere with the analysis.[101]

Methods of analysis of trace quantities of PCDD/PCDF have improved markedly in recent years and this permits a significant reduction in their limits of detection. They are, for example (depending on the isomers), for samples of air 0.01–0.05 [105] and 0.1–1 pg m^{-3},[103] sludges (for TCDD) 0.1 ng kg^{-1},[126] drinking water 1 pg $litre^{-1}$,[109] blood plasma 0.003–0.02 [144] and 1.25 pg $litre^{-1}$ (for TCDD),[119] adipose tissue 2–10 ng kg^{-1},[139] milk 0.1–0.5 [147] and 0.2–0.4 ng $litre^{-1}$.[145]

Table 5. Molecular ions of polychlorinated compounds present in environmental test samples and possibly interfering in the mass spectrometric analysis of PCDD and PCDF.[101]

Molecular ions (m/z, M^+, M^-) (chlorination)	Compounds					
	PCBF	PCB	PCN	PCT	PCDPE	PCP
Mono-	186	188	162	265	204	236
Di-	220	222	196	298	238	270
Tri-	254	256	230	332	272	304
Tetra-	288	290	264	366	306	338
Penta-	322	324	298	400	340	372
Hexa-	356	358	332	434	374	406
Hepta-	390	392	366	468	408	440
Octa-	424	426	400	502	442	474
Nona-	—	460	—	536	476	508
Deca-	—	494	—	570	510	542

6. Safety techniques when carrying out analyses

It is necessary to take special safety measures when carrying out analyses because of the extreme toxicity of many isomers of PCDD and PCDF. These begin at sample taking and end with the decontamination of the waste water after carrying out analyses.[164]

When taking samples of environmental objects precautions depend strongly on the concentrations of PCDD/PCDF presumed to be present. In the case of strongly contaminated places of sampling and of the samples themselves special safety measures must be applied which are linked with the organisation of decontamination areas, special clothing, etc. In uncontaminated places test samples of PCDD/PCDF are collected with the same precautions as when taking samples of other highly toxic xenobiotics for analysis.

The selected samples are carefully packed and transferred to the station laboratory. Each test sample of soil and sludge in glass beakers with teflon screw lids is placed in a plastic container which is itself placed in a metal container with kieselguhr. Biological samples are wrapped 2–3 times in aluminium foil and transferred in the frozen state in foam-plastic containers. Water samples in glass bottles are first placed in a plastic container with kieselguhr which is then put into a metal container with kieselguhr.

All work must be carried out in the station laboratory in a fume cupboard. Liquid waste water must be mixed with methanol or ethanol and irradiated with UV light at a wavelength > 290 nm for several days. Residues of samples coming out of a chromatograph and GC/MS sample effluent must be passed through a column of activated charcoal or bubbled through a trap of oil or high boiling alcohol.

Working surfaces and equipment in a station laboratory are sampled periodically with pieces of filter paper to determine their cleanliness. This is considered satisfactory at a content of 1 μg 2,3,7,8-TCDD in the wipe sample. At >1 μg the location must be cleaned and at >10 μg there is a threat of contamination and consequently working places must be cleaned meticulously.

The problems arising after the accumulation in nature of a large number of highly toxic chlorine–containing xenobiotics oblige mankind to solve a most complex analytical problem, viz. to develop methods for the quantitative and qualitative determination at the ppt level of polycyclic mixtures in various natural objects. This requires highly pure, and frequently ultrapure, organic solvents, inorganic acids, adsorbents and other chemical reagents, expensive high purity standards for compounds and the isomers to be determined, chromatographs with capillary columns and gas chromatograph-mass spectrometers of high (6000–10000) resolution with modern systems for processing the results obtained. For the safe determination of complex mixtures of dioxins and furans exceptional cleanliness of laboratory premises is required, to the level for carrying out genetic engineering work, there are very stiff requirements for safety techniques and high qualifications are needed for the scientific and service personnel. The combination of these necessary conditions has a strong influence on the cost of analysis of the first sample, from several tens for determining insecticides to $US 3000 for the isomerically specific determination of dioxins and furans in biological samples.

Nonetheless the large material outlay in developed countries on the bulk analysis of polychlorinated compounds, particularly the most toxic isomers of dioxins, furans and biphenyls in environmental samples, and, which is very important, in human milk, adipose tissue and blood plasma is considered necessary since these xenobiotics are one of the main sources of progressing immunodeficiency of higher organisms, similar in character to the immunodeficiency caused by the AIDs virus.

In the last five years the industrially developed countries have joined in the need to increase the sensitivity of methods of determining analytically down to a level of 1 ppq (pg kg^{-1}) for 2,3,7,8-TCDD. Only in this case does it seem possible to assess admissible levels for these xenobiotics for the developing foetus and the newborn and to solve many practically important problems including a check on the purity of childrens' food. There is no doubt that the solution of this problem reduces in principle to new technology in ultramicroanalysis.

References

1. *Metodicheskie Ukazaniya po Opredeleniyu Toksichnykh Zagryaznyuyushchikh Veshchestv v Morskoi Vode na Fonovom Urovne* (Methodological Information on the Determination of Toxic Contaminants in Sea Water at the Background Level), No. 45, Gimiz, Moscow, 1982, p. 17
2. *Metodicheskie Ukazaniya po Khimicheskomu Analizu Raspresenennykh Vod Morskikh Ust'evykh Oblastei Rek i Epokontinental'nykh Morei* (Methodological Information on the Chemical Analysis of Fresh Water of Sea Estuary Regions of Rivers and Apocontinental Seas), No. 46, Gimiz, Moscow, 1984, p. 44
3. M Mohnke, K H Rohde, L Brugmann, P Franz *J. Chromatogr.*, 1986, **364** 327

4. A R Reich, J L Perkins, G Cutter *Environ. Toxic. Chem.*, 1986, **5** 725
5. D B Martin, W A Hartman *J. Assoc. Off. Anal. Chem.*, 1985, **68** 712
6. *Metodicheskie Ukazaniya po Opredeleniyu Zagryaznyayushchikh Veshchestv v Morskikh Donnykh Otlozheniyakh* (Methodological Information on the Determination of Contaminants in Sea Bed Sediments), No. 43, Gimiz, Moscow, 1979, p. 6
7. *Vremennye Metodicheskie Rekomendatsii po Kontrolyu Zagryazneniya Pochv* (Provisional Methodological Recommendations on the Control of Contamination of Soils), Gimiz, Moscow, 1983, p. 8
8. C P Kaushik, M K K Pillai, A Raman, H C Agarwal *Water Air Soil Pollut.*, 1987, **32** 63
9. T F Bidleman, U Wideqvist, B Jansson, R Soederlund *Atmos. Environ.*, 1987, **21** 641
10. M K Saiki, C J Schmitt *Arch. Environ. Contam. Toxicol.*, 1986, **15** 357
11. G L LeBel, D T Williams *J. Assoc. Off. Anal. Chem.*, 1986, **69** 451
12. W H Stickel, L F Stickel, R A Dyrland, D L Hughes *Environ. Monit. Assessm.*, 1984, **4** 113
13. S P Havera, R E Duzan *Bull. Environ. Contam. Toxic.*, 1986, **36** 23
14. H A Price, R L Welch, R H Scheel, L A Warren *Bull. Environ. Contam. Toxic.*, 1986, **37** 1
15. D T Williams, G L LeBel, E Junkins *J. Toxic. Environ. Health*, 1984, **13** 19
16. R Dommarco, A Di Muccio, I Camoni, B Gigli *Bull. Environ. Contam. Toxic.*, 1987, **39** 919
17. *Metody Opredeleniya Mikrokolichestv Pestitsidov v Produktakh Pitaniya, Kormakh i Vneshnei Srede* (Methods of Determining Micro Amounts of Pesticides in Foodstuffs, Fodder and the External Environment), Kolos, Moscow, 1983, p. 5
18. H V Zubillaga, J L Sericano, A E Pucci *Water Air Soil Pollut.*, 1987, **32** 43
19. F Mangani, G Crescentini, P Palma, F Bruner *J. Chromatogr.*, 1988, **452** 527
20. G A Junk, J J Richard *Anal. Chem.*, 1988, **60** 451
21. E Nozoozian, F A Maris, M W F Nielen, et al. *J. High Resolution Chromatogr. Chromatogr. Commun.*, 1987, **10** 17
22. M Suzuki, M Morimoto *J. High Resolution Chromatogr. Chromatogr. Commun.*, 1986, **9** 692
23. H Steinwandter *Fresinius' Z. Anal. Chem.*, 1987, **327** 309
24. A Ambrus, H P Their *Pure Appl. Chem.*, 1986, **58** 1035
25. N Ahmad, R S Marold *J. Assoc. Off. Anal. Chem.*, 1986, **69** 581
26. C J Schmidt, J L Zajicek, M A Ribick *Arch. Environ. Contam. Toxic.*, 1985, **14** 225
27. A Di Mussio, M Rizrica, A Ausili, et al *J. Chromatogr.*, 1988, **456** 143
28. C Fooken, W Butte *Chemosphere*, 1987, **16** 1301
29. M P Seymour, T M Jefferies, A J Floyd, L J Natariani *Analyst (London)*, 1987, **112** 427
30. *Metody Opredeleniya Mikrokolichestv Pestitsidov* (Methods of Determining Micro Amounts of Pesticides), Meditsina, Moscow, 1984, p. 129
31. P De Voogt, J C Klamer, H Govers *J. Chromatogr.*, 1986, **363** 407
32. J Mes, D J Davies, D Turton *Bull. Environ. Contam. Toxic.*, 1982, **28** 97
33. A M Gillespie, S M Walters *J. Liquid Chromatogr.*, 1986, **9** 2111
34. F M Gretch, J D Rosen *J. Assoc. Off. Anal. Chem.*, 1987, **70** 109
35. A H Roos, A J Van Munsteren, F M Nab, L G M T Tuinstra *Anal. Chim. Acta*, 1987, **196** 95
36. S M Prinsloo, P R De Beer *J. Assoc. Off. Anal. Chem.*, 1987, **70** 878
37. J P Thome, Y Vandaele *Int. J. Environ. Anal. Chem.*, 1987, **29** 95
38. G Petrick, D E Schulz, J C Duinker *J. Chromatogr.*, 1988, **435** 241
39. B Bush, L A Shane, M Wahlen, M P Brown *Chemosphere*, 1987, **16** 733
40. F A Maris, E Noroozian, R R Otten, et al. *J. High Res. Chromatogr. Chromatogr. Commun.*, 1988, **11** 197
41. R Masse, D Martineau, L Tremblay, P Beland *Arch. Environ. Contam. Toxic.*, 1986, **15** 567
42. S Tanabe, A Subramanian, H Hidaka, R Tatsukawa *Chemosphere*, 1986, **15** 343
43. S Tanabe, R Tatsukawa, D J H Phillips *Environ. Poll.*, 1987, **47** 41
44. S Tanabe, N Kannan, T Wakimoto, R Tatsukawa *Int. J. Environ. Anal. Chem.*, 1987, **29** 199
45. P Fernandes, C Porte, D Barcelo, et al. *J. Chromatogr.*, 1988, **456** 155
46. L Maack, W C Sonzogni *Arch. Environ. Contam. Toxic.*, 1988, **17** 711
47. T R Schwartz, D L Stalling, C L Rice *Environ. Sci. Technol.*, 1987, **21** 72
48. A El Nabashi, B Heinzow, H Kruse *Arch. Environ. Contam. Toxic.*, 1987, **16** 689
49. J De Boer *Chemosphere*, 1988, **17** 1803
50. J De Boer *Chemosphere*, 1988, **17** 1811
51. W A Neidmann *Chemosphere*, 1986, **22** 363
52. J Mes, D Turton, et al. *Int. J. Environ. Anal. Chem.*, 1987, **28** 197
53. J Mes *Environ. Res.*, 1987, **44** 213
54. G Eduljee, K Badsha, L Price *Chemosphere*, 1985, **14** 1371
55. D L Swackhamer, D E Armstrong, *Environ. Sci. Techno.*, 1986, **20** 879
56. C S Cleaser, A R Fernandes *Chemosphere*, 1986, **15** 499
57. A L Alford-Stevens, W L Budde, T A Bellar *Anal. Chem.*, 1985, **57** 2452
58. B J Brownawell, J W Farrington *Geochim. Cosmochim. Acta*, 1986, **50** 157
59. A L Alford-Stevens *Anal. Chem.*, 1986, **58** 2014
60. K Ballschmiter, M Zell *Fresenius' Z. Anal. Chem.*, 1980, **302** 20
61. E D Pellizzari, M A Moseley, S D Cooper *J. Chromatogr.*, 1985, **334** 277
62. K Ballschmiter, W Schafer, H Buchert *Fresenius' Z. Anal. Chem.*, 1987, **326** 253
63. L E Slivon, J E Gebhart, T L Hayes, et al. *Anal. Chem.*, 1985, **57** 2464
64. A L Alford-Stevens, T A Bellar *Anal. Chem.*, 1986, **58** 2022
65. R D Voyksner, J T Bursey, T W Pack, R L Porch *Anal. Chem.*, 1986, **58** 621
66. M D Mullin, C M Pochini, et al. *Environ. Sci. Technol.*, 1984, **18** 468
67. J C Duinker, D E Schulz, G Petrick *Anal. Chem.*, 1988, **60** 478

68. J C Duinker, D E Schulz, G Petrick *Mar. Poll. Bull.*, 1988, **19** 19
69. G Schomburg *J. High Res. Chromatogr. Chromatogr. Commun.*, 1985, **8** 395
70. F I Onuska, K A Terry *J. High Res. Chromatogr. Chromatogr. Commun.*, 1986, **9** 671
71. S Safe, L Safe, M Mullin *J. Agric. Food. Chem.*, 1985, **33** 24
72. J Mes, D Turton, D Davies, et al. *Int. J. Environ. Anal. Chem.*, 1987, **28** 197
73. B Leece, M A Denomme, R Towner, et al. *Toxic. Environ. Health*, 1985, **16** 379
74. R Hemann, A Kettrup *Chemosphere*, 1987, **16** 527
75. A J Cessna, R Grover *J. Agric. Food. Chem.*, 1985, **13** 504
76. H B Lee *J. Assoc. Off. Anal. Chem.*, 1986, **69** 557
77. H B Lee, A S Y Chau *J. Assoc. Off. Anal. Chem.*, 1983, **66** 1023
78. D F Gurka, F L Shore, S T Pan *J. Assoc. Off. Anal. Chem.*, 1987, **70** 889
79. M J Bertrand, A W Ahmed *Anal. Chem.*, 1987, **59** 1302
80. D F Gurka *J. Assoc. Off. Anal. Chem.*, 1986, **69** 970
81. K Abrahamsson, T M Xie *J. Chromatogr.*, 1983, **279** 199
82. T M Xie, K Abrahamsson *Environ. Sci. Technol.*, 1986, **20** 457
83. J Paasivirta, J Knuutinen, M Knuutila, et al. *Chemosphere*, 1988, **17** 147
84. B B Sithole *J. Assoc. Off. Anal. Chem.*, 1986, **69** 466
85. J Paasivirta, J Knuutinen, P Maatela, et al. *Chemosphere*, 1988, **17** 137
86. H B Lee *J. Assoc. Off. Anal. Chem.*, 1987, **70** 1003
87. M Remberger, P A Hynning, A H Neilson *Environ. Toxic. Chem.*, 1988, **7** 795
88. J Paasivirta, P Klein, M Knuutila, et al. *Chemosphere*, 1987, **16** 1231
89. J Folke, J Birklund *Chemosphere*, 1986, **15** 895
90. J Hajslova, V Kocourek, I Zemanova, et al. *J. Chromatogr.*, 1988, **439** 307
91. O H Masi, W M Gulick, *J. High Res. Chromatogr. Chromatogr. Commun.*, 1987, **10** 647
92. H B Lee, A S Y Chau *J. Assoc. Off. Anal. Chem.*, 1983, **66** 1029
93. H B Lee, L D Weng, A S Y Chau *J. Assoc. Off. Anal. Chem.*, 1984, **67** 1086
94. M J Bertrand, S Stefanidis *J. Chromatogr.*, 1986, **354** 331
95. P Trippel, W Maasfeld, A Kettrup *Int. J. Environ. Anal. Chem.*, 1985, **23** 97
96. C Borra, A Di Corcia, M Marchetti, R Samperi *Anal. Chem.*, 1986, **58** 2048
97. H Malissa, C Szolgyenyi, K Winsauer *Fresenius' Z. Anal. Chem.*, 1985, **321** 17
98. K Ballschmiter, H Buchert, R Niemczyk, et al. *Chemosphere*, 1986, **15** 901
99. R J Kociba *Solving Hazardous Waste Problems* (Edited by J H Exsner), Am. Chem. Soc., Washington, 1987, p. 54
100. C Rappe *Environ. Sci. Technol.*, 1984, **18** 78A
101. C Rappe, M Nygren, H R Buser *Application of New Mass Spectrometry Technique in Pesticide Chemistry* (Edited by J Rosen), Wiley, New York, 1987, p. 60
102. R E Clement, H M Tosine *Mass Spectrometry Rev.*, 1988, **7** 593
103. M Oehme, S Mano, A Mikalsen, P Kirschmer *Chemosphere*, 1986, **15** 607
104. R M Smith, P W O'Keefe *Anal. Chem.*, 1986, **58** 2414
105. C Reppe, L O Kijeller *Chemosphere*, 1988, **17** 3
106. K Scheide, R P Kuna, F Wurst *Chemosphere*, 1985, **14** 913
107. C L Haile, R B Blair, J S Stanley, et al. *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (Edited by L H Keith et al.), Butterworth Publ., Boston, 1985, p. 439
108. B J Fairless, D I Bates, J Hudson, et al. *Environ. Sci. Technol.*, 1987, **21** 550
109. G L LeBel, D T Williams, J J Ryan, B P Y Lau *Chlorinated Dioxins and Dibenzofurans in Perspective* (Edited by C Rappe et al.), Lewis Publ., Michigan, 1986, p. 329
110. P O'Keefe, C Meyer, R Smith, et al. *Chemosphere*, 1986, **15** 1127
111. J W Anderson, G H Herman *Chemosphere*, 1985, **14** 1115
112. H Heida, K Olie *Chemosphere*, 1985, **14** 919
113. R L Harless, E O Oswald, R G Lewis, et al. *Chemosphere*, 1982, **11** 193
114. D W Kuehl, E Durhan *Environ. Int.*, 1984, **10** 45
115. S W Kaczmar, M J Zabik, F M D'ltri *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (Edited by L H Keith et al.), Butterworth Publ., Boston, 1985, p. 103
116. M Oehme, P Furst, C Kruger, et al. *Chemosphere*, 1988, **17** 1291
117. P W Albro, W B Crummett, A E Dupuy, et al. *Anal. Chem.*, 1985, **57** 2717
118. M Ono, T Wakimoto, R Tatsukawa, Y Masuda *Chemosphere*, 1986, **15** 1629
119. D G Patterson, L Hampton, C R Lapeza, et al. *Anal. Chem.*, 1987, **59** 2000
120. S Facchetti, A Leoni *Application of Mass Spectrometry to Trace Analysis* (Edited by S Facchetti), Elsevier, New York, 1982, p. 215
121. P O'Keefe, D Hilker, C Meyer, et al. *Chemosphere*, 1984, **13** 849
122. R M Smith, P W O'Keefe, C Meyer, et al. *Environ. Sci. Technol.*, 1983, **17** 6
123. L M Smith, D L Stalling, J L Johnson *Anal. Chem.*, 1984, **56** 1830
124. D R Thielen, G Olsen *Anal. Chem.*, 1988, **60** 1332
125. J S Stanley *Biomed. Environ. Mass Spectrom.*, 1988, **17** 27
126. E H G Evers, K C M Ree, K Olie *Chemosphere*, 1988, **17** 2271
127. R A Niemann, W C Brumley *Anal. Chem.*, 1983, **55** 1497
128. W A Korfmacher, G F Moler, et al. *Chemosphere*, 1984, **13** 669
129. J J Ryan *J. Assoc. Off. Anal. Chem.*, 1983, **66** 700
130. R G Heath, R L Harless, M L Gross, et al. *Anal. Chem.*, 1986, **58** 463
131. J Lawrence, F Onuska *Chemosphere*, 1986, **15** 1085
132. Y Tondeur, P W Albro, J R Hass, et al. *Anal. Chem.*, 1984, **56** 1344
133. P O'Keefe, C Meyer, D Hilker, et al. *Chemosphere*, 1983, **12** 325
134. D G Patterson, J S Holler, C R Lapeza, et al. *Anal. Chem.*, 1986, **58** 705
135. D W Kuehl *Chemosphere*, 1987, **16** 657
136. M Van den Berg *Chemosphere*, 1986, **15** 693
137. H Beck, K Eckart, et al. *Biomed. Environ. Mass Spectrom.*, 1988, **16** 161
138. R J Norstrom *Int. J. Environ. Anal. Chem.*, 1986, **23** 267
139. J J Ryan, A Schecter, W F Sun, R Lizotte *Chlorinated Dioxins and Dibenzofurans in Perspective* (Edited by C Rappe et al.), Lewis Publ., Michigan, 1986, p. 3

140. D W Kuehl, P M Cook, A R Batterman, et al. *Chemosphere*, 1987, **16** 667
141. T H Umbreit, E J Hesse, M A Gallo *Science*, 1986, **232** 231
142. B K Afghan, J Carron, P D Goulden, et al. *Can. J. Chem.*, 1987, **65** 1086
143. J S Stanley, K E Boggess, J Onstot, et al. *Chemosphere*, 1986, **15** 1605
144. M Nigren, M Hansson, M Sjostrom, et al. *Chemosphere*, 1988, **17** 1663
145. G Lindstrom, C Rappe *Chemosphere*, 1988, **17** 921
146. L L Lamparski, T J Nestrik *Anal. Chem.*, 1980, **52** 2045
147. H Beck, K Eckart, M Kellert, et al. *Chemosphere*, 1987, **16** 1977
148. T Sakuma, N Gurprasad, S D Tanner, et al. *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (Edited by L H Keith et al.), Butterworth Publ., Boston, 1985, p. 139
149. J J Ryan, D T Williams, B P Y Lau *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (Edited by L H Keith et al.), Butterworth Publ., Boston, 1985, p. 205
150. Y Tondeur, W N Niederhut, J E Campana, S R Missler *Biomed. Environ. Mass. Spectrom.*, 1987, **14** 449
151. H R Buser, C Rappe, P A Berqvist *Environ. Health Persp.*, 1985, **60** 293
152. H R Buser, C Rappe *Anal. Chem.*, 1984, **56** 442
153. T Muzer, F D Hileman, R W Noble, J J Brooks *Anal. Chem.*, 1983, **55** 104
154. D S Waddell et al. *Biomed. Environ. Mass Spectrom.*, 1987, **14** 457
155. M Swerev, K Ballschmiter *Anal. Chem.*, 1987, **59** 2536
156. U Riehle, J Ehmann *Fresenius' Z. Anal. Chem.*, 1988, **331** 821
157. M D Hale, F D Hileman, T Mazer et al. *Anal. Chem.*, 1985, **57** 640
158. H Kuroki, K Haraguchi, Y Masuda *Chemosphere*, 1984, **13** 561
159. M Oehme, P Kirschmer *Anal. Chem.*, 1984, **56** 2754
160. F L Onuska *J. High Res. Chromatogr. Chromatogr. Commun.*, 1988, **11** 9
161. P Schmidt, M D Muller *J. High Res. Chromatogr. Chromatogr. Commun.*, 1987, **10** 548
162. N H Mahle, L A Shadoff *Biomed. Environ. Mass Spectrom.*, 1982, **9** 45
163. G R B Webster, K Olie, O Hutzinger *Mass Spectrometry in Environmental Sciences* (Edited by F W Karasek et al.), Plenum Press, 1985, p. 257
164. C Elly *Chlorinated Dioxins and Dibenzofurans in the Total Environment* (Edited by L H Keith et al.), Butterworth Publ., Boston, 1985, p. 311