

Rapid Determination of Persistent Organic Pollutants (POPs) Using Accelerated Solvent Extraction (ASE)

INTRODUCTION

The United Nations Environmental Program (UNEP) has been implemented in an effort to combat the release of selected persistent organic pollutants (POPs). POPs are found in environmental samples such as soils, sludges, solid and semisolid waste, and sediments. POPs are also found in biological samples such as human breast milk, and fish tissue. UNEP is interested in eliminating POPs from the environment because these compounds are considered toxic, carcinogenic, and mutagenic, and degrade slowly in the environment, posing a threat to the global environment. The following compounds are listed by UNEP to be POPs:

- **Pesticides:**

Aldrin, Chlordane, DDT, Dieldrin, Endrin, Heptachlor, Mirex, and Toxaphene

- **Industrial chemicals:**

Hexachlorobenzene, and PCB (polychlorinated biphenyl)

- **Chemical by-products (Dioxins):**

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD and PCDF)

Accelerated Solvent Extraction (ASE) is equivalent to U.S. EPA Methods 3540, 3541, 3550, and 8150 for the extraction of organochlorine pesticides (OCPs), organophosphorous pesticides (OPPs), semivolatiles or base neutral acids (BNAs), chlorinated herbicides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). ASE complies with

U.S. EPA Method 3545A for these compounds. ASE is an extraction technique that significantly streamlines sample preparation. This technique uses extraction solvents at elevated temperatures and pressure to increase the kinetics of the extraction process. The high pressure allows the solvent to be used above its boiling point, keeping it in a liquid state, and thus decreases the amount of time and solvent required to extract the desired analyte from the sample matrix. ASE replaces extraction techniques such as Soxhlet, sonication, and wrist-shaker with equivalent or better results.

This application note describes methods and results for extraction of the POPs listed above, with tables comparing ASE to traditional extraction methods.

EQUIPMENT

Dionex ASE 200 Accelerated Extractor with Solvent Controller (P/N 048765)*

Use either:

22 mL Stainless Steel Extraction Cells (P/N 048764)

11 mL Stainless Steel Extraction Cells (P/N 048765)

33 mL Stainless Steel Extraction Cells (P/N 048766)

Cellulose Filters (P/N 049458)

Collection Vials 60 mL (P/N 048784) or Collection Vials 40 mL (P/N 048783)

Analytical Balance (to read to nearest 0.0001 g or better)

ASE Prep DE (diatomaceous earth) (P/N 062819)

**ASE 150 and 350 can be used for equivalent results*

SOLVENTS

Hexane

Dichloromethane

Acetone

Toluene

(All solvents are pesticide-grade or equivalent and available from Fisher Scientific.)

EXTRACTION CONDITIONS

Pesticides and PCBs (8081/8082)

Solvent: Hexane/acetone (1:1), (v/v)

Temperature: 100 °C

Pressure: 1500 psi*

Static Time: 5 min

Static Cycles: 1–2

Flush: 60%

Purge: 60–120s

Hexachlorobenzene (8270)

Solvent: Dichloromethane/acetone (1:1), (v/v)

Temperature: 100 °C

Pressure: 1500 psi

Static Time: 5 min

Static Cycles: 1–2

Flush: 60%

Purge: 60–120 s

Dioxins (PCDD and PCDF) (8290)

Solvent: Toluene (100%) or toluene/acetic acid (5%, v/v) if HCl pretreatment currently used

Temperature: 175–200 °C

Pressure: 1500 psi

Static time: 5–15 min

Static cycles: 2–3

Flush: 60–70%

Purge: 60–120 s

**Pressure studies show that 1500 psi is the optimum extraction pressure for all ASE applications.*

SAMPLE INFORMATION AND EXTRACTION PROCEDURES

Pesticide Sample Information

Spiking concentrations ranged from 5 to 250 µg/kg. All spiked soils were prepared and certified by ERA (Environmental Resource Associates, Arvada, Colorado, USA). Spiked samples were extracted both by the ASE 200 system and by a Perstorp Environmental Soxtec® (automated Soxhlet). Matrix blanks, spikes, and spike duplicates were included for the low-level spikes; matrix spikes were included for all other concentrations. Collected extracts from the ASE 200 were approximately 13–15 mL from the 11 mL extraction cells and approximately 26–30 mL from the 22 mL cells. Extracts can be further cleaned up or directly analyzed depending on the extent of interfering coextractables. For the examples shown in the application note, extracts were analyzed by SW-846 Method 8080. All extractions and analytical work were performed by an independent testing laboratory, Mountain States Analytical, Inc. (Salt Lake City, Utah, USA.)

Pesticide Extraction Procedure

Mix sample thoroughly, especially composite samples. Dried sediment, soil, and dry waste samples should be ground or otherwise subdivided to pass through a 1-mm sieve. Introduce sufficient sample into the grinding apparatus to yield at least 10–20 g after grinding. Air-dry the sample at room temperature for 48 h in a glass tray or on hexane-cleaned aluminum foil, or dry the sample by mixing with ASE Prep DE (diatomaceous earth) until a free-flowing powder is obtained. Air-drying is not appropriate for the analysis of the more volatile organochlorine pesticides (e.g., the BHCs), because of losses during the drying process. For sediment and soils (especially gummy clay) that are moist and cannot be air-dried because of loss of volatile analytes, mix 5–10 g of sample with an equal amount of ASE Prep DE in a small beaker using a spatula. Use this approach for any solid sample that requires dispersion of the sample particles to ensure greater solvent contact throughout the sample mass.

Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise separated to allow mixing and maximum exposure of the sample surfaces for the extraction. If grinding of these materials is preferred, the addition and mixing of ASE Prep DE with

the sample (1:1, w/w) may improve grinding efficiency. The professional judgment of the analyst is required for handling such difficult matrices.

Place a cellulose disk at the outlet end of the extraction cell. Weigh approximately 10 g of each sample into 11-mL extraction cells, or approximately 20 g into 22-mL cells. For samples mixed with ASE Prep DE, transfer the entire contents of the beaker to the extraction cell. Surrogate spikes and matrix spikes may be added to the appropriate sample cells.

Place extraction cells into the autosampler tray and load the collection tray with the appropriate number (up to 24) of 60-mL, precleaned, capped vials with septa. Set the method conditions on the ASE 200 system and initiate the run.

PCB Sample Information

Sewage sludge was obtained from the Fresenius Institute (Tanusstein, Germany). Oyster tissue samples were obtained from the National Oceanographic and Atmospheric Administration (NOAA) Laboratory (Seattle, Washington, USA). The river sediment is a standard reference material, SRM 1939 (National Institute of Science and Technology, Gaithersburg, Maryland, USA). Contaminated soil used in this study was a certified reference material (CRM911-050) purchased from Resource Technology Corporation (Laramie, Wyoming, USA).

PCB Extraction Procedure

Samples should be dried and ground. Before filling the cell, a cellulose disk should be placed in the outlet end of the cell. Samples that contain water (greater than 10%) should be mixed in equal proportions with ASE Prep DE.

Quantification of Sewage Sludge, Oyster Tissue, and River Sediment

Sample extracts from ASE were prepared for analysis by passing through silver nitrate/sulfuric acid loaded silica gel and alumina columns, followed by concentration to 1 mL for GC analysis. PCB analyses were performed by gas chromatography with ECD using a 30-m × 0.25-mm i.d., Rtx-5 (Restek, Bellefonte, Pennsylvania, USA) or equivalent column. Injector and detector were maintained at 300 °C. The GC oven was programmed from 100–300 °C at 10 °C/min following a 5-min hold. External standards were used for calibration.

Quantification of Soil (CRM911-050)

PCB analyses of the soil extracts were performed according to U.S. EPA SW-846 Method 8080. The ASE 200 extracts were diluted to 25 mL prior to analysis by GC. Injection was through a split/splitless injector in a GC with dual-electron capture detectors. Two capillary columns, a 30-m × 0.53 mm i.d. DB-608 and a 30-m × 0.53 mm i.d. DB-1701 (J&W Scientific, Folsom, California, USA) provided primary and confirmation data, respectively. Both columns were joined with a fused-silica Y connector (Restek). The remaining part of the Y was connected to a 5 m section of deactivated 0.53 mm i.d. fused-silica capillary tubing that acted as a guard column. The end of this guard column was inserted into the GC injector. Dual confirmation of the analytes was achieved with a single 5 µL injection. The injector was maintained at 220 °C and both detectors were operated at 320 °C. The oven was programmed from 60–200 °C at 28 °C/min after a 1 min hold, then 265 °C at 10 °C/min with a hold of 20.5 min. Helium was used as the carrier gas at a linear velocity of approximately 30 cm/s.

Hexachlorobenzene Sample Information

Spiking concentrations ranged from 250 to 12,500 µg/kg for the semivolatiles (BNA compounds). All spiked soils were prepared and certified by ERA (Environmental Resource Associates). Samples were ground to 100–200 mesh (150–75 µm). Wet samples were mixed with either ASE Prep DE (10 g sample to 10 g ASE Prep DE), or air-dried. After grinding, a weighed sample was transferred to either a 11 or 22 mL extraction cell.

Spiked samples were extracted both by the ASE 200 system and by a Perstorp Environmental Soxtec® (automated Soxhlet). Extracts were analyzed by SW-846 Method 8270A.

Note: All extractions and analytical work were performed by Mountain States Analytical, Inc. (Salt Lake City, Utah, USA). Matrix blanks, spikes, and spike duplicates were included for the low-level spikes; matrix spikes were included for all other concentrations.

Hexachlorobenzene Extraction Procedure

The procedure used in this application note follows the detailed method as described under the U.S. EPA SW-846 Method 3545A.

Mix sample thoroughly, especially composite samples. Dried sediment, soil, and dry waste samples should be ground or otherwise subdivided to pass through a 1-mm sieve. Introduce a sufficient amount of sample into the grinding apparatus to yield at least 10–20 g after grinding. Air-dry the sample at room temperature for 48 h in a glass tray or on hexane-cleaned aluminum foil, or dry the sample by mixing with ASE Prep DE until a free-flowing powder is obtained. Air-drying is not appropriate for the analysis of the more volatile organochlorine pesticides (e.g., the BHCs), or the more volatile of the semivolatile organics because of losses during the drying process.

Gummy, fibrous, or oily materials not amenable to grinding should be cut, shredded, or otherwise separated to allow for mixing and maximum exposure of the sample surfaces for extraction. If grinding of these materials is preferred, the addition and mixing of ASE Prep DE with the sample (1:1, w/w) may improve grinding efficiency.

For sediment and soils (especially gummy clay) that are moist and cannot be air-dried because of loss of volatile analytes, mix 5–10 g of sample with an equal amount of ASE Prep DE in a small beaker using a spatula. Use this approach for any solid sample that requires dispersion of the sample particles to ensure greater solvent contact throughout the sample mass.

Place a cellulose disk into the extraction cell. Weigh approximately 10 g of each sample into an 11-mL extraction cell or approximately 20 g into a 22 mL extraction cell. Transfer the entire contents of the beaker to the extraction cell. Surrogate spikes and matrix spikes may be added to the appropriate sample cells.

Place extraction cells into the autosampler tray and load the collection tray with the appropriate number (up to 24) of 40-mL, precleaned, capped vials with septa. Set the method conditions on the ASE 200 system and initiate the run.

Collected extracts will be approximately 13–15 mL from the 11-mL extraction cells and 26–30 mL from the 22-mL size cells. The extract is now ready for cleanup or analysis depending on the extent of interfering coextractables.

Dioxins (PCDD and PCDF) Sample Information

Two different sample sets were investigated: one from Germany that included chimney brick, urban dust, and fly ash, and a second from Canada that included four sediment samples. A sediment sample (EC-2) containing high ng/kg levels (ppt) of PCDDs and PCDFs was obtained from the National Water Research Institute (867 Lakeshore Road, P.O. Box 5050, Burlington, Ontario, L7R 4A6, Canada). A low-level sediment sample (HS-2) was obtained from the National Research Council Institute for Marine Biosciences (1411 Oxford Street, Halifax, Nova Scotia, B3H 3Z1, Canada). Both samples are being investigated as potential standard reference materials and were used as received. Two other sediment samples, Parrots Bay and Hamilton Harbor, were extracted. Both of these samples have high levels of coextractable materials.

Dioxins (PCDD and PCDF) Extraction Procedure

Table 1 lists the conditions used for the extraction of the chimney brick and urban dust by Soxhlet and ASE.

The procedure for the extraction of fly ash was slightly different than the procedure for the other matrices. Before solvent extraction, the samples were treated with 6 M HCl for 30 min and then rinsed thoroughly with distilled water. The extractions by both Soxhlet and ASE were then performed as listed in Table 1. One additional set of extractions was performed on fresh fly ash samples. Instead of pretreatment with HCl, 5% (v/v) glacial acetic acid was added to the toluene for the ASE extraction. All other conditions were held constant.

Table 1. Extraction Conditions for Chimney Brick and Urban Dust

Condition	Soxhlet	ASE
Sample Size	4–10 g	4–10 g
Solvent	Toluene, 250 mL	Toluene 15 mL
Temperature	<111 °C	150 °C
Pressure	Atmospheric	1500 psi
Time	18 h	5-min heatup
Cycles		5-min static, 2 or 3 cycles
Analytical	GC/MS	GC/MS

Quantitation and Sample Cleanup

Cleanup on the chimney brick, urban dust, and fly ash sample extracts was performed by using a chromatographic column packed with multiple layers of silica gel and alumina, in accordance with the German method VDI 3499.

Extracts from the sediment samples were cleaned up using a dual-stage open column chromatography procedure consisting of modified silica and alumina stationary phases.

Samples were further cleaned using an automated HPLC carbon-based method to remove diphenylether interferences. Complete details of the analytical procedure are available in reference 1.

Analysis by GC/MS and GC/MS/MS

Extracts of the chimney dust, urban dust, and fly ash samples were analyzed by GC-LRMS. The column used for the chromatography was a 60-m × 0.25-mm i.d. × 0.15-μm film thickness J&W DB-Dioxin column.

Sediment sample extracts were analyzed by GC/MS/MS. All extracts were separated using a 60-m × 0.25-mm i.d. × 0.25-μm film thickness J&W DB-5 fused-silica capillary column.

Standards

An internal standard solution containing 10 reference compounds, including $^{13}\text{C}_{12}$ -2,3,7,8- T_4CDD was used for the chimney dust, urban dust, and fly ash samples. No cleanup standard was used. Samples were reconstituted with a recovery standard solution (100 μL) containing $^{13}\text{C}_{12}$ -1,2,3,4- T_4CDD at 25 ng/mL.

For the sediment samples, standard PCDD/PCDF mixtures were prepared from stock solutions obtained from either Cambridge Isotope Laboratories, Inc. or Wellington Laboratories. The internal quantitation standard contained 15 $^{13}\text{C}_{12}$ -2,3,7,8-substituted PCDDs and PCDFs. The compounds used are those congeners listed in the data tables. Following extraction, the samples were spiked with a cleanup standard ($^{37}\text{C}_{14}$ -2,3,7,8- T_4CDD) to differentiate between losses occurring at the extraction and cleanup stages. Prior to injection, the samples were reconstituted with a recovery standard solution (10 μL) containing $^{13}\text{C}_{12}$ -1,2,3,4- T_4CDD and $^{13}\text{C}_{12}$ -1,2,3,7,8,9- H_6CDD at 100 pg/μL in nonane.

RESULTS AND DISCUSSIONS

Pesticides

Table 2 and 3 shows examples of extraction of selected environmental samples, including both spiked and incurred samples, are shown. These examples illustrate the effectiveness of the ASE technique in obtaining recoveries of analytes equivalent to Soxtec. Tables 2 and 3 summarize the results of this study for chlorinated pesticides spiked at three different levels, in three different soil types.

Table 2. Average Recovery of Pesticides from Three Soil Types^a—ASE Compared to Automated Soxhlet

Pesticide	Average Recovery (% of Soxhlet)
Heptachlor	88.0
Aldrin	94.9
Gamma Chlordane	99.5
Alpha Chlordane	102.0
Dieldrin	101.2
Endrin	97.2
p,p'-DDT	74.9

^a Averages from extraction of sand, loam, and clay soils.

Table 3. Average RSD (%) for Chlorinated Pesticides

Matrix	ASE	Automated Soxhlet
Clay	5.0	9.7
Loam	7.8	6.2
Sand	12.0	10.1

PCBs

Results from extractions of sewage sludge, oyster tissue, river sediment, and soil are shown in Tables 4 through 7. These tables show the average recoveries and RSDs (%) for PCB congener content of these matrices. Recoveries for all compounds with the exception of one (PCB 153 from the river sediment) are above 77% of the certified or Soxhlet comparison values. Interferences in the river sediment extract prevented quantification of two low-molecular-weight PCB congeners (PCB 28 and PCB 52).

Table 4. PCB Recoveries from Sewage Sludge^a		
PCB Congener	Average Recovery, n = 6 (as % of Soxhlet)	RSD (%)
PCB 28	118.1	2.5
PCB 52	114.0	4.7
PCB 101	142.9	7.4
PCB 153	109.5	5.8
PCB 138	109.6	3.9
PCB 180	160.4	7.5

^a Analyte concentration range: 160–200 µg/kg per component

Table 5. PCB Recovery from Oyster Tissue^a		
PCB Congener	Average Recovery, n = 6 (as % of Soxhlet)	RSD (%)
PCB 28	90.0	7.8
PCB 52	86.9	4.0
PCB 101	83.3	1.5
PCB 153	84.5	3.5
PCB 138	76.9	3.0
PCB 180	87.0	4.3

^a Analyte concentration 50–150 µg/kg per component

Table 6. PCB Recovery from River Sediment (SRM 1939)^a		
PCB Congener	Average, n = 6 (as % of Soxhlet)	RSD (%)
PCB 101	89.2	3.7
PCB 153	62.3	4.1
PCB 138	122.1	2.3
PCB 180	111.5	5.9

^a Analyte concentration 170–800 µg/kg per component

Table 7. Recovery of Arochlor 1254 from Soil (CRM911-050)	
Run Number	Arochlor Found (µg/kg)
1	1290.0
2	1365.8
3	1283.4
4	1368.6
Average	1327.0 (99.0%)
RSD	3.51%

The results demonstrate the effectiveness of ASE as a sample preparation method. ASE provides extracts with minimal solvent usage and significant time reduction compared to other extraction methods. Results are comparable to the traditional Soxhlet extraction method. ASE meets the requirements for PCB analysis as described in U.S. EPA SW-846 Method 3545A.

Hexachlorobenzene

This application note shows extraction examples selected environmental samples. This study illustrates the effectiveness of the ASE technique in obtaining recoveries of hexachlorobenzene equivalent to Soxtec. Tables 8 and 9 summarize the results for Hexachlorobenzene at three different spiking levels, in three different soil types, that were extracted according to the method presented. ASE recoveries and RSD (%) values were all within the range expected from Soxhlet extractions.

Table 8. Average Recovery of BNA from Three Soil Types^a—ASE Compared to Automated Soxhlet^a	
BNA Target Compound	Average Recovery (% of Soxhlet)
Hexachlorobenzene	93.7

^a Averages from extraction of sand, loam, and clay soils

Table 9. Average RSD (%) for BNA for Three Soil Types		
Matrix	ASE	Automated Soxhlet
Clay	9.1	9.6
Loam	16.1	15.2
Sand	13.4	17.1

Dioxins (PCDD and PCDF)

Ground Chimney Brick and Urban Dust

Table 10 shows the results from the ground chimney brick and urban dust as selected congeners and as the total of the isomers. The toxicity equivalent is calculated by adding the weighted factors of each isomer's toxicity. One is calculated according to a formula from the North Atlantic Treaty Organization (NATO) and the other is from the German health organization BgVV. The results show that ASE is equivalent to the Soxhlet method with respect to recovery of these compounds.

Fly Ash

Table 10 lists the results from the extractions of the fly ash. The units for this sample are µg/kg because the sample was so highly contaminated. ASE results are equivalent to those from Soxhlet extractions when the HCl/water pretreatment was used.

High-Level Sediment Samples

Table 10 presents a comparison of average results for the Soxhlet and ASE methods for the high-level sediment sample (EC-2). The data compare very favorably.

The data for sample HS-2 also shows a favorable comparison trend (Table 10).

**Table 10. Comparison of Soxhlet vs ASE—
Total^a Polychlorinated Dibenzo-*p*-dioxins**

Sample Matrix	Soxhlet (ng/kg)	ASE (ng/kg)
Chimney Brick	8040	8170
Urban Dust	1110	1159
Fly Ash (µg/kg)	93,000	107,900
Sediment (EC-2)	6750	6840
Sediment (HS-2)	11,731	12,783
Hamilton Harbor Sediment	4283	4119
Parrots Bay Sediment	2836	2444

^aTotal of tetra-, penta-, hexa-, hepta-, and octachlorodibenzo-*p*-dioxins

Highly Contaminated Sediment Samples

The ASE technique was also evaluated with two sediment samples containing high levels of coextractables and oil (Table 10). Aliquots of these samples were taken from a larger container as quantitatively as possible, but were not nearly as homogeneous as the rigorously prepared reference materials. Generally, the data compare favorably between ASE and Soxhlet for the recovery of PCDDs and PCDFs from these heavily contaminated sediments.

CONCLUSION

The data shows that ASE is essentially equivalent to classical extraction procedures such as Soxhlet for the extraction of POPs from environmental matrices. In addition to being equivalent to Soxhlet, ASE can perform the extractions in a fraction of the time and with much less solvent.

SUPPLIERS

Fisher Scientific, 2000 Park Lane, Pittsburgh, PA 15275-1126 USA, Tel: 800-766-7000, www.fishersci.com.

Varian, Inc. Corporate Headquarters, 3120 Hansen Way, Palo Alto, CA 94304-1030 USA, Tel: 650-213-8000, www.varianinc.com.

National Water Research Institute, 867 Lakeshore Road, Burlington Ontario L7R 4A6 Canada.

National Research Council Institute for Marine Biosciences, 1411 Oxford Street, Halifax Nova Scotia, B3H 3Z1 Canada.

Sigma-Aldrich Chemical Company, 3050 Spruce St., St. Louis, MO 63103 USA, Tel: 800-325-3010, www.sigmaaldrich.com.

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