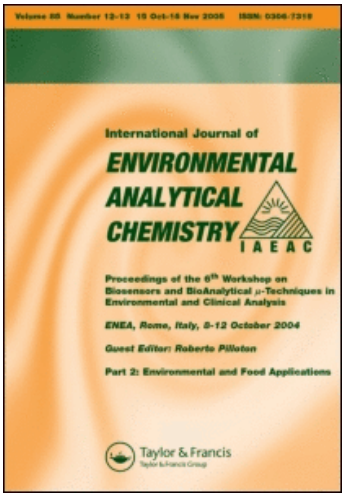

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NEW CLEANUP PROCEDURE FOR DETERMINING POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN LIPOPHILIC MATRICES

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A new cleanup procedure for determining polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in naturally occurring adipose matrices is suggested. The procedure can be used for analyzing butter, human and cow's milk, eggs, and meat. Quality of sample purification is better than that in the standard procedure, and the recovery of PCDD and PCDF internal standards is within the range 50-80%. The time it takes for sample preparation is shortened, and the consumption of solvents and sorbents is decreased. The FAS-MD activated carbon, which was especially prepared for the extraction of dioxins and related compounds, was used in this work.

Keywords: Polychlorinated dibenzo-p-dioxin (PCDD); polychlorinated dibenzofuran (PCDF); cleanup procedure; lipophilic matrix; chromatography; activated carbon

INTRODUCTION

Environmental monitoring for polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) is presently of great importance. A wide variety of matrices should be analyzed to obtain the pattern of distribution for dioxins in particular regions. Problems associated with these analyses involve a great

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number of specialized laboratories required and high expenses of performing these examinations. A two-stage analysis may be advantageous in this case. At the first stage, integral lipophilic matrices, which characterize the pollution of a whole region, are analyzed. If positive results are obtained (that is, concentrations of the substances under examination are higher than maximum permissible concentrations), the next stage is required. At the second stage, an additional number of matrices should be analyzed, and the sources of dioxins should be identified.

Various environmental samples can be used as the integral matrices to characterize the presence of dioxins, for example, bottom sediments near the sources of waste water, snow and, to a lesser extent, air. However, snow and air reflect only a current pollution level, and they are inadequate for evaluating severe contamination with PCDDs and PCDFs. Bottom sediments provide more information, but sampling is rather random and cannot be adequate for assessing pollution of the whole region.

Biological adipose matrices such as cow's milk, butter, meat, and eggs are more suitable for measuring total pollution levels. Human milk is one of the most informative matrices for the evaluation of human exposure to PCDDs and PCDFs.

At the first stage, the analysis of matrices should be simple, rapid, inexpensive, and, if possible, automatic, and it should provide an opportunity to locate and outline contaminated areas.

The most commonly used cleanup procedures for milk,[1-3] eggs,[4] and meat[5-7] result in high quality of cleaning, but they are very sophisticated, time consuming, and expensive (great amounts of chemicals are needed). Thus, they cannot be used as the test methods of rapid monitoring for dioxins. Moreover, these methods are difficult to automate, because it is necessary to evaporate toluene from the samples after cleaning on a carbon column.[8]

The aim of this work was to develop an inexpensive rapid procedure of sample preparation for lipophilic matrices at the first stage of the environmental monitoring for dioxins.

EXPERIMENTAL

Reagents and Apparatus

Solvents and reagents were of Pesticide Grade (Burdick & Jackson) or reagent grade (Russia). Kieselgel 60 (Merck), Al_2O_3 (Bio-Rad), and FAS-MD activated carbon (Institute of Physical Chemistry, Russian Academy of Sciences) were used as sorbents. PCDDs labeled with $^{13}C_{12}$ and $^{13}C_6$ (CIL) served as standard

compounds. All samples were spiked with a standard mixture containing 2 ng of $^{13}C_{12}$-labeled tetrachlorodibenzo-p-dioxin (TCDD), 2 ng of $^{13}C_{12}$-labeled pentachlorodibenzo-p-dioxin (PCDD), 4 ng of $^{13}C_{12}$-labeled hexachlorodibenzo-p-dioxin (HxCDD), 4 ng of $^{13}C_{12}$-labeled heptachlorodibenzo-p-dioxin (HpCDD), and 4 ng of $^{13}C_{12}$-labeled octachlorodibenzo-p-dioxin (OCDD).

An ULTRA-TURRAX 125 (Janke & Kunkel IKA-Labortechnik) homogenizer was used. Determinations by GC—MS were performed on a Varian 3400 gas chromatograph and a Finnigan MAT HSQ-30 high-resolution mass spectrometer. A 30-m HP-17 fused silica capillary column with a film thickness of 0.25 mm was employed.

Extraction

Butter and pork fat

A 10-g sample of butter or fat tissue was dissolved in 200 ml of a hexane-acetone (1:1) mixture and spiked with the standard mixture. The solution was shaken with 20-50 g of ammonium sulfate for 5-10 min, settled, and left to stand for 2 h. The organic layer was decanted and filtered.

Cow's and human milk

A 150-ml sample of milk was spiked with the standard mixture. Then, 150 ml of acetone and 150 ml of hexane were added to the sample; the contents were homogenized for 3 min; 105 g of ammonium sulfate was added to the homogenate, and the mixture was shaken till all ammonium sulfate was dissolved. When the layers were separated (5 min), the sediment was filtered off through a glass filter with the use of a slight vacuum. The sediment was washed with two 10-ml portions of hexane-acetone (1:1). These hexane-acetone portions were added to the main extract. The organic layer (about 300 ml) was separated with the use of a separatory funnel, and 20 ml of acetone was added. The aqueous layer was discarded.

Eggs

An egg was weighed; water was added up to 150 g, and the contents were homogenized for 5 min and then spiked with labeled standards. The sample was homogenized with 150 ml of acetone and 150 ml of hexane for 3 min, and 105 g of ammonium sulfate was added. The mixture was shaken until all ammonium sulfate was dissolved. After phase separation (about 1 h), the organic layer was decanted. The remainder was centrifuged (3000 rpm; 5 min), and the organic

layer was decanted. The organic extracts were combined, and 20 ml of acetone was added. The total volume of the organic extract was about 300 ml.

Meat (beef and poultry)

Meat was minced and stirred. A 100-g sample of meet was spiked with the standard mixture. To the sample were added 150 ml of acetone and 150 ml of hexane, and the contents were homogenized for 3 min until a uniform homogenate was formed. Then, 50 g of ammonium sulfate was added. The mixture was shaken until all ammonium sulfate was dissolved. When the layers were separated, the sediment was filtered off through a glass filter with the use of a slight vacuum. The sediment was washed with two 10-ml portions of hexane-acetone (1:1). These hexane-acetone extracts were added to the main extract. The organic layer was separated with the use of a separatory funnel, and 20 ml of acetone was added. The aqueous layer was discarded.

Carbon Microcolumn

A carbon microcolumn (Figure 1) was packed with 20 mg of FAS-MD activated carbon on 200 mg of Celite 545. The microcolumn was prepared from a glass pipette (i.d. 3.5 mm). A length of the sorbent layer was 2.5 cm. The sorbent was fixed at two ends by glass fiber plugs. The extract was applied to the carbon column under a pressure of 0.5-1.5 atm. The column was washed with 20 ml of hexane-acetone (1:1). PCDDs and PCDFs were eluted in the reverse direction with 5 ml of toluene at 80°C. The eluent was heated with the use of a microheater made from a 8200-Ω PEV-15 resistor (Russia). The hexane-acetone extract was evaporated, and the total lipids were determined gravimetrically.

Multilayer Column

The toluene eluate of a sample (5 ml) was mixed with 45 ml of hexane. This solution was applied to a multilayer column (i.d. 10 mm) packed with 1 cm^3 of neutral silica, 1 cm^3 of 40% H_2SO_4 on silica (60%), 1 cm^3 Na_2SO_4, and 1 cm^3 of K_2SiO_3 (from top to bottom). Then the column was rinsed with 50 ml of hexane. The fractions were joined.

Neutral Alumina Column

Weighed portions of Al_2O_3 (4 g) were placed into glass ampoules and calcined at 450°C for 17 h. The ampoules were cooled to 200°C and sealed. They were opened immediately before preparing columns.

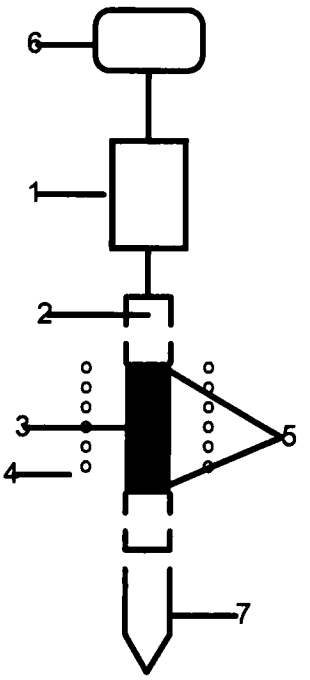

FIGURE 1 Carbon microcolumn for preconcentration of dioxins from extracts of lipophilic matrices: (1) reservoir, (2) glass pipette (i.d. 3.5 mm), (3) FAS-MD activated carbon (20 mg) on Celite 545, (4) microheater, (5) glass-fiber plugs, (6) compressor, and (7) collector.

A 100-ml portion of the eluate from the previous stage was applied to the column packed with 4 g of neutral alumina. Interfering substances were sequentially eluted with 20 ml of hexane and 30 ml of methylene chloride-hexane (5:95). Then, analytes were eluted with 50 ml of methylene chloride-hexane (50:50). $^{13}C_6$-TCDD was added as an recovery standard, and the solution was concentrated and transferred into a small sample vial containing 5 ml of tridecane as a keeper. The solvents were removed by passing a nitrogen flow.

RESULTS AND DISCUSSION

We suggest a new cleanup procedure for determining PCDDs and PCDFs in the naturally occurring adipose matrices: milk, meat, eggs, and butter (Figure 2). Extraction with an acetone-hexane solution (1:1) is performed according to the procedure described in[9] followed by saturation with ammonium sulfate. In so

doing, organic compounds are salted out into the organic layer, and proteins are denatured.

We found that distribution coefficients of PCDDs and PCDFs in the acetone-hexane-water and acetone-hexane-milk systems (1:1:1) were significantly increased upon saturation with inorganic salts, and the extraction was complete.

Calcium chloride and sodium sulfate were also examined as the saturating agents. However, ammonium sulfate gave the best results. The sediments formed in the course of processing various matrices differed in structure; however, they can be easily decanted, filtered off, or centrifuged in all cases. PCDDs and

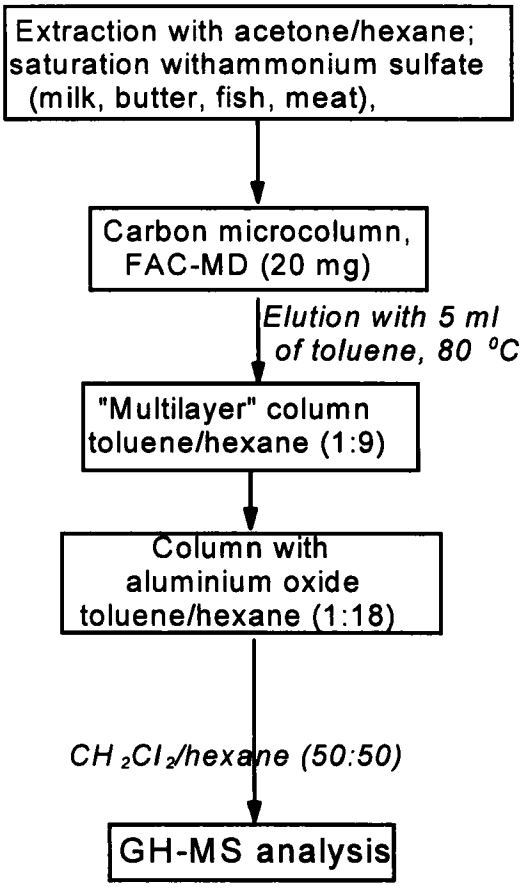

FIGURE 2 Cleanup procedure for determining PCDDs and PCDFs in lipophilic matrices.

PCDFs were separated from an acetone-hexane extract with the use of a carbon microcolumn.

A new type of carbon microcolumns was described previously[10, 11] (see Figure 1). The previous microcolumns were packed with 20 mg of AX-21 (Anderson Development Co., USA) or FAS (Neorganika, Elektrostal, Russia) activated carbon with a specific surface area of 651 or 171 m^2/g, respectively, as determined from benzene adsorption isotherms. These columns make it possible to separate PCDDs and PCDFs from acetone-hexane (1:1) extracts of adipose matrices (300-400 ml) without preliminary evaporation and cleaning. Lipids and impurities of nonplanar structure were removed at the first step of the treatment of extracts. Thus, the consumption of sorbents and solvents and time necessary for the next steps of cleaning were significantly reduced. However, 80 ml of toluene was needed for eluting all dioxin congeners from a carbon microcolumn packed with AX-21 or FAS. Figures 3a and 3b present the desorption diagrams for model solutions of 2,3,7,8-TCDD and OCDD with the use of toluene heated to 80°C. The stage of toluene evaporation makes the cleanup procedure rather long; moreover, automation is impossible in this case.

We tested different types of activated carbon and examined the relationship between the sorption characteristics and physicochemical properties. The best results were obtained with the FAS-MD activated carbon. FAS-MD is highly effective microporous carbon sorbent produced of furfurol copolymer (a specific surface area was 1100 m^2/g as determined from benzene adsorption isotherms). It was specially prepared for the sorption of dioxins. A microcolumn with FAS-MD completely sorbed PCDDs and PCDFs from 200-300 ml of acetone-hexane solutions. Only 5 ml of toluene was required to completely elute PCDDs and PCDFs from the column (Figure 3c).

For additionally cleaning, the extract was passed through a small multilayer column packed with 1 cm^3 of neutral silica, 1 cm^3 of silica with 40% H_2SO_4, and 1 cm^3 of K_2SiO_3. We found that the cleaning on the multilayer column was effective in a toluene-hexane solution (1:9). All dioxins were retained on the column packed with Al_2O_3 from a toluene-hexane solution (1:18); thus, the evaporation of toluene was unnecessary.

The advantages of the procedure suggested are illustrated below.

The recovery of PCDDs and PCDFs from sample matrices

The recovery was evaluated by the use of labeled standards. Table I summarizes the experimental data obtained. The average recovery is in the range 60-80%, which is in good agreement with the results obtained by conventional procedures.[2, 12, 13]

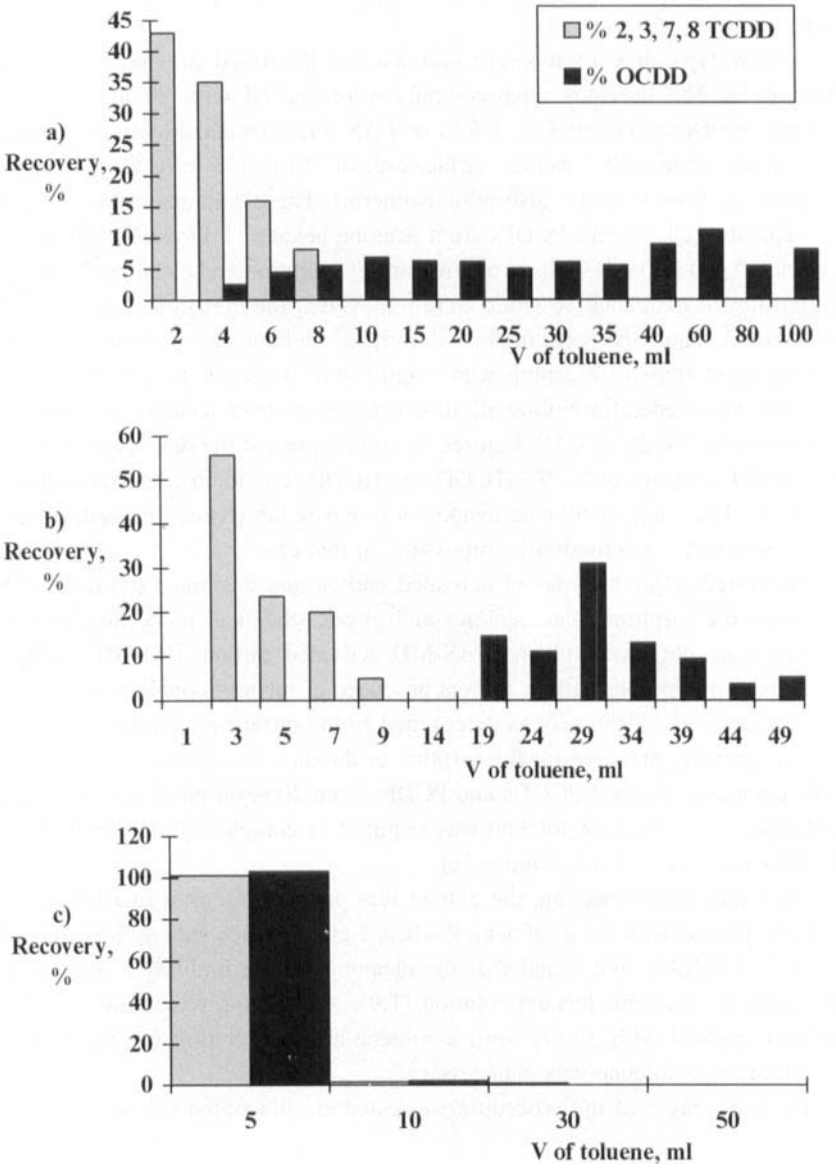

FIGURE 3 Desorption diagrams for 2,3,7,8-TCDD and OCDD from carbon columns packed with: (a) AX-21, (b) FAS, and (c) FAS-MD activated carbon. Desorption was performed in the reverse direction with toluene heated to 80°C.

TABLE I Recovery of labeled PCDDs from various adipose matrices

Recovery, % Standardcompound	Butter					Milk					Eggs				Poultry	Beef	Pork	n	s	ΔX		
$^{13}C_{12}$-TCDD	61	61	80	57	71	61	48	45	61	49	74	65	84	50	59	100	94	63	53	62	15	7.5
$^{13}C_{12}$-PCDD	100	91	55	81	74	61	70	58	31	71	70	61	47	39	65	83	70	61	52	68	16.9	8.5
$^{13}C_{12}$-HxCDD	50	57	67	75	58	38	45	82	74	50	39	42	42	70	97	82	102	97	80	66	20.9	10.0
$^{13}C_{12}$-HpCDD	104	102	50	93	90	88	104	93	72	55	104	72	72	64	84	85	104	80	96	85	16.9	8.1
$^{13}C_{12}$-OCDD	90	93	66	97	103	59	69	86	65	57	78	56	50	59	63	82	99	90	88	76	16.9	8.1

n is the average persenage; s is the root-mean-square deviation; and ΔX is the confidence interval

Cleaning quality

Selective separation of PCDDs and PCDFs with 5 ml of toluene from initial extracts is possible due to the use of a carbon microcolumn packed with the FAS-MD activated carbon. Only small quantities of sorbents in multilayer and alumina columns are required for the subsequent cleaning. The columns were not overloaded, and a breakthrough of contaminants was impossible. The cleaning quality is better than that of the standard method,[15] because a lot of concomitant organic interferents are removed from the final extract.

Apparatus

The cleanup procedure does not require evaporation steps at each stages and can be completely automated. Of special equipment, only a compressor or a gas cylinder with a pressure regulator are used.

Efficiency and economy

The extraction and cleanup steps of the procedure suggested are more rapid than those in the standard procedure. These steps take only 6 h on the average. Sorbent and solvent consumption is significantly decreased. Table II compares the requirements for time and chemicals used in the suggested and standard[15] procedures.

Safety

Elimination of a step of liquid-liquid extraction with the use of concentrated acids and bases is an advantage of the procedure described. Unlike the conventional procedure for milk,[1-3] the extraction procedure suggested is performed without diethyl ether.

CONCLUSIONS

We suggested to perform a two-stage analysis for monitoring PCDD and PCDF concentrations in the environment and to use lipophilic matrices at the first stage of this monitoring. The most convenient lipophilic matrices are milk, butter, eggs, and meet.

A general-purpose cleanup procedure for determining PCDDs and PCDFs in lipophilic matrices was suggested. Extraction from lipophilic tissues was performed with the use of acetone-hexane solutions followed by saturation of the system with ammonium sulfate.

TABLE II Comparison between sample preparation procedures for determining PCDDs and PCDFs in 10-g samples of lipophilic matrices

Parameters	Standard procedure	Suggested procedure
Volume of the carbon column (ml)	4-5	0.3-0.4
Volume of toluene required for the elution from the carbon column (ml)	200	5
Time required for the elution from the carbon column (h)	5-6	2-3
Volume of the multilayer column (ml)	300	6
Volume of a solvent required for the elution from the multilayer column (ml)	1500-2000	100
Time required for the elution from the multilayer column (h)	>24	1
Total time required for the analysis (h)	>24	5-6

A new type of activated carbon (FAS-MD) used allowed us to elute all PCDD and PCDF congeners from a carbon microcolumn with a small volume of toluene. The stage of toluene evaporation was eliminated. The elimination of the evaporation stage makes the procedure easy to automate.

The procedure suggested simplifies the sample preparation for determining dioxins in adipose matrices; it is more rapid and less expensive. Lesser amounts of sorbents and toxic solvents are required.

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