Supercritical Fluid Extraction of Organochlorine Pesticides in Eggs

Walter Fiddler,*,† John W. Pensabene,† Robert A. Gates,† and Dan J. Donoghue‡

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture,
600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, and Center for Veterinary Medicine, Division of
Animal Research, U.S. Food and Drug Administration, 8401 Muirkirk Road, Laurel, Maryland 20708

The efficacy of supercritical fluid extraction (SFE) for the recovery of 16 common organochlorine pesticides (OCPs) from liquid whole eggs was investigated by employing supercritical carbon dioxide (SC-CO_2) without the use of a solvent modifier to minimize interfering coextractives. The OCPs tested included aldrin; α-, β-, δ-, and γ-BHCs; *p,p′*-DDD, -DDE, and -DDT; dieldrin; endosulfans I, II, and sulfate; endrin; endrin aldehyde; heptachlor; and heptachlor epoxide. The SFE conditions were as follows: 10000 psi (680 bar), 40 °C, SC-CO_2 flow rate of 3.0 L/min with an extraction time of 40 min for a total of 120 L of CO_2. The OCPs were trapped off-line in an SPE cartridge containing Florisil and then eluted by an acetone/hexane mixture and analyzed by gas chromatography-electron capture detection (GC-ECD). Recovery studies were carried out on homogenized eggs fortified at the 0.05, 0.10, and 0.20 ppm levels. At the lowest level, 0.05 ppm, recoveries ranged from 81.8 to 108.3%, with CVs $<$ 9.8%. All recoveries were significantly higher than those obtained by an AOAC/FDA solvent extraction method. Eggs containing incurred endosulfan I were also effectively extracted by SFE. This study suggests that the application of SFE for the extraction of OCPs from eggs will result in significant savings in analysis time and lower solvent use and disposal costs compared to conventional solvent extraction procedures.

Keywords: *Supercritical fluid extraction; SFE; organochlorine pesticides; OCPs; chicken eggs*

INTRODUCTION

There are increasing public concerns over the presence of organochlorine pesticides (OCPs) in the environment and food supply due to their known toxicity, including the carcinogenicity of some members of this group of compounds. Additionally, these concerns include their immunosuppressive (Thomas, 1995) and hormonal disruption (Colborn et al., 1996) activities. Monitoring for OCPs is an essential part of any surveillance and compliance program, especially in fat-containing foods because they may contain these fat-soluble compounds. Even in countries where the use of these pesticides has been banned or restricted, high levels of DDT and other OCPs persist in the environment and consequently in the human food chain (Anonymous, 1995). With an estimated production of >65 billion chicken eggs per year (Eggs for Industry, 1997), egg consumption represents a significant potential source of human exposure to pesticide residues by virtue of the average diet. This is why the OCP analysis of eggs is essential. Currently, the FDA uses a conventional acetonitrile solvent extraction–Florisil column chromatographic method described in the *Association of Official Analytical Chemists' Official Methods of Analysis* (AOAC, 1996) and the FDA's *Pesticide Analytical Manual* (FDA, 1994). Because this method is time-consuming and requires large amounts of solvent, more efficient methodology is needed.

The Environmental Protection Agency (EPA, 1991) requires the promotion of practices that reduce environmental pollution. Executive Order 12856 (*Federal Register*, 1993) specifically requested that federal agencies take the lead in developing pollution prevention plans to reduce their release of toxic chemicals into the environment. This included source reduction of organic solvents in federal laboratories, especially for those solvents requiring disposal as hazardous waste. This makes the development of solvent-free or solvent-sparing isolation methods for residue analysis a worthwhile objective. Supercritical fluid extraction (SFE) has the potential to be an alternative to many solvent extraction methods that are currently used.

The solubilizing property of SC-CO_2 has been reported to be equivalent to that of benzene (Janda et al., 1989), making it effective for the extraction of most OCPs, because this class of pesticides is relatively nonpolar. Reports of the isolation of OCPs from environmental samples, such as soils, sludge, and sediments, are extensive; generally temperatures up to 65 °C and pressures up to 6000 psi are used. More often than not, a small percentage of methanol modifier or cosolvent is used to increase OCP recoveries by overcoming analyte–matrix interactions, not for solubility purposes. However, information on the application of SFE for the isolation of OCPs from fat-containing foods is not extensive. In this case, the analyte–matrix effect is not as important; the critical isolation step is separating the fat-soluble OCPs from the coextracted fat. This can be done in a number of different ways, either when SC-CO_2 is employed with a modifier or even when supercritical fluoroform is used (Ashraf-Khorassanie et al., 1996). King et al. (1993) retained 99+% of the chicken fat on neutral alumina located in the extraction vessel

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* Author to whom correspondence should be addressed [fax (215) 233-6559; e-mail wfiddler@arserrc.gov].

† Eastern Regional Research Center.

‡ Center for Veterinary Medicine, Division of Animal Research.

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with CO_2 at 250 atm and 50 °C. Hopper (1997) used a unique SFE system operated at 4000 psi and 60 °C with 3% acetonitrile-modified CO_2 to obtain good recoveries for 43 of 62 nonpolar to moderately polar OCPs and organophosphates (OPPs) in different fats. These pesticides were separated from the fats by retaining the fats at 4000 psi on an in-line C_{18} bonded silica column in a 95 °C oven directly attached to the outlet of the SFE instrument. For these types of samples, many of the OCPs were effectively extracted at 6000 psi and 60 °C, without a modifier. The acetonitrile modifier was needed primarily for the OPPs. An automated SFE and in-line cleanup version of this method was found to be applicable to 86 nonpolar to moderately polar OCPs and OPPs in samples containing <17% fat and <70% moisture (Hooper, 1998). Although the use of a polar solvent modifier increases the range of analytes that can be extracted, there are disadvantages. This obviously includes increasing the amount of potentially interfering coextractives that have to be removed later by a cleanup step. The use of modifier could also interfere with analyte collection by deactivating the sorbent or causing breakthrough if the OCPs are trapped on a sorbent either in-line or off-line.

The excellent OCP recoveries obtained by Hopper (1997) in oils, and the effective SFE extraction of incurred heptachlor, dieldrin, and endrin from chicken fat at 10000 psi and 80 °C (Snyder et al., 1993), suggested that OCPs could be readily analyzed in a fat-containing sample such as eggs. There is only one report on the specific use of SFE for the isolation of OCPs from eggs. Wigfield et al. (1996) proposed its use as a alternative screening method to the solvent−time intensive Soxhlet extraction/gel permeation chromatography/Florisil Sep-Pak cleanup procedure employed by Agriculture and Agri-Food Canada (1993). In it, they carried out the SFE of 22 OCPs from fortified eggs at 250 atm and 60 °C, with and without methanol modifier. At the 0.05 ppm spiking level, recoveries from 50 to 112% were obtained.

In this paper, we report the results of using SFE with only SC-CO_2 at 10000 psi (680 atm) and with off-line SPE cartridge trapping for the extraction of 16 OCPs, designated in EPA Method 608, from whole eggs fortified at three different concentrations. Also, a comparison between SFE and a solvent extraction procedure was carried out with both fortified eggs and eggs containing incurred endosulfan I.

EXPERIMENTAL PROCEDURES

Materials. The pesticides were purchased from AccuStandard (New Haven, CT) individually, neat. Hydromatrix (Celite 566, sieved at 30−40 mesh to remove fines; Applied Separations, Allentown, PA), acetone and hexane (Burdick & Jackson Brand, Baxter Health Care, Muskegon, MI), and silica gel (60−100 mesh, Aldrich Chemical Co., Milwaukee, WI) were used without further purification. Florisil (60−100 mesh, Aldrich Chemical Co.) was washed with acetone, filtered, and dried in a vacuum oven for 4 h at 150 °C before use. SFC-grade CO_2 without helium headspace (Scott Specialty Gas, Plumsteadville, PA) was used for the extraction. For the spiked recovery studies, eggs were obtained from a local supermarket. The eggs were removed from their shells, the yolk and white thoroughly mixed, and the eggs stored in a −85 °C freezer until used. The eggs were thawed in a 4 °C refrigerator and kept for 2 weeks before being discarded.

For the normally incurred pesticide feeding study, performed at an FDA, CVM laboratory, six laying hens were given a single daily dose of 6 mg of endosulfan I (orally by gelatin capsule) on 2 consecutive days. Twenty-four eggs were collected on days 2−7. Eggs were also collected during the corresponding period from four control nondosed laying hens fed a pesticide-free diet. Composite samples from each group were homogenized, shipped to the Eastern Regional Research Center, and then placed in frozen storage at −85 °C until analyzed.

Supercritical Fluid Extraction. One gram of liquid egg was weighed into a 100 mL beaker and fortified with 50, 100, or 200 μL of the mixed OCP standard (1.0 μg/mL each in MeOH). After 15 min was allowed for the sample to equilibrate, 4.0 g of Hydromatrix was added to the beaker and the sample stirred with a glass rod until a dry, free-flowing mixture was obtained. Into a high-pressure (10000 psi) 24 mL extraction vessel (Keystone Scientific, Bellefonte, PA), was added a plug of polypropylene wool (Aldrich Chemical Co.), 2 g of silica gel, and another plug of polypropylene wool. The sample mixture was added to the extraction vessel, followed by a plug of polypropylene wool. Finally, 2 g of Hydromatrix was added to the extraction vessel, topped by another plug of polypropylene wool. All of the material added to the extraction vessel was firmly tamped. For installation in the SFE oven, the end of the extraction vessel packed with silica gel was designated the top, as illustrated in Figure 1.

The extractions were carried out in a two-vessel parallel extractor (Applied Separations). The description and setup of the extractor are described elsewhere (Maxwell et al., 1992). The system was operated under the following conditions: oven, 40 °C; micrometering valve, 110 °C; extraction pressure, 10000 psi (680 atm); flow rate of expanded gas, 3.0 L/min; total flow, 120 L. The pesticides were collected off-line in 6 mL SPE cartridges (Applied Separations) containing 1.25 g of Florisil. After completion of the extraction, the pesticides were eluted from the off-line Florisil SPE cartridges with 8 mL of a 10% acetone in hexane solution. The extract was concentrated to 1.0 mL for quantitation.

Additional Cleanup Procedure. For the removal of the occasional interfering peak at 32 min, an empty 6 mL cartridge was packed with 2.5 g of prewashed Florisil and topped with a sintered glass disk. The 1.0 mL hexane/acetone egg extract was reduced to ≤0.1 mL with a gentle stream of nitrogen, and the volume was brought up to 1.0 mL with hexane and then quantitatively transferred to the Florisil column. The tube was rinsed with ~5 mL of petroleum ether/diethyl ether (65:35), and it and an additional 25 mL were added to the top of the Florisil column. The eluate was collected in a Kuderna−Danish concentrator apparatus and concentrated to 1.0 mL.

Gas Chromatography. Analyses were carried out on a Hewlett-Packard HP 6890 series gas chromatograph (Palo Alto, CA) equipped with an electron capture detector. An H-P autosampler was fitted to the inlet for splitless injection. The detector temperature was 260 °C, and the injector inlet was set at 220 °C. A 30 m × 320 μm i.d. SGE BPX5, 0.25 μm film thickness, capillary column was employed with He carrier gas maintained at a constant flow rate of 2.5 mL/min by electronic pressure control. The oven was programmed from 110 to 145 °C at 20 °C/min and then from 145 to 220 °C at 2 °C/min. Each sample injection (2 μL) was bracketed between injections of a standard of OCPs. The standard's responses were averaged to calculate the OCP recoveries. Overall, the limit of sensitivity or detectability was estimated to be 0.001 ppm (1.0 ppb), based on a signal-to-noise ratio >2:1. Instrumental conditions could be varied to increase the sensitivity and decrease the minimum level of detection, should it be needed. For the spiked recovery study, five replicate analyses were performed at each concentration level. The detector response was found to be linear over the range 1 ppb to 20 ppm (relative to a 1 g sample).

Statistical Analysis. The data for each individual pesticide in the fortified recovery study were analyzed by two-way ANOVA with replication to determine whether there were significant ($p < 0.05$) differences in recovery between the SFE and the AOAC/FDA procedures or among the different levels of fortification. The data for the normally incurred endosulfan I study was analyzed by the two-way ANOVA with replication, where the two factors examined for significant ($p < 0.05$) differences were the time of egg collection and the two

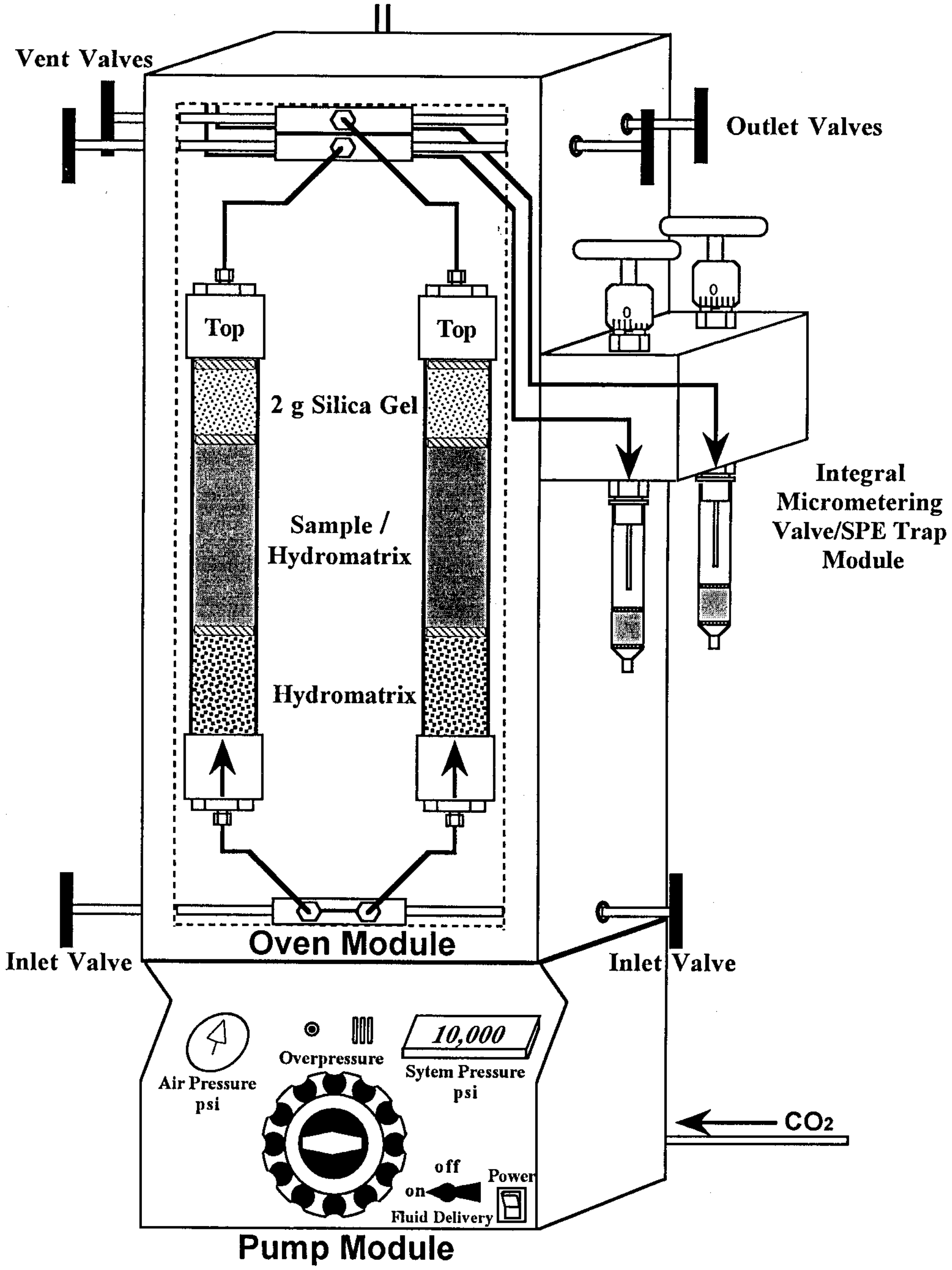

Figure 1. Schematic of the supercritical fluid extractor.

analytical procedures. Data were analyzed using the Statistical Analysis System PC software (SAS, 1985), and interpreted according to the methods of Snedecor and Cochran (1979) and Youden and Steiner (1975).

RESULTS AND DISCUSSION

Because of their high lipophilicity, OCPs are accumulated and stored in an animal's adipose tissue. In egg-laying chickens, these compounds are translocated into the lipid-containing egg yolk. For eggs and other fat-containing foods, the SC-CO_2 extract contains a mixture of lipids and pesticides that often require one or more post-SFE cleanup operations before GC analysis. One reported approach used to separate the analytes from coextracted fat is to trap the analytes in-line on a sorbent placed at the top of the extraction vessel or in a second vessel, while allowing the fat to pass through. Alley and Lu (1995) successfully applied this technique for the isolation of PCBs from spiked chicken eggs and fish. They showed that silica gel, 3-aminopropyl, and Florisil were better than aluminum oxide, C_{18}, and cyano sorbents for this purpose. In contrast to their SFE conditions (6000 psi and 65 °C), under our extraction conditions (10000 psi and 40 °C) we found that this in-line trapping-separation method gave low recoveries for some of the OCPs.

Because of the problems associated with in-line pesticide trapping, we needed an alternate collection/separation technique. Other investigators have successfully trapped the fat on an in-line sorbent during SFE while collecting the analytes in the sample off-line after decompression. France et al. (1991) took this approach to extract pesticides by trapping the fat on a packed bed of either alumina or silica gel. Although there are many reported advantages to trapping the fat in-line, complete fat retention on the sorbent is not always assured, especially at the higher pressures. We found this to be especially true for the extraction conditions we employed to extract the OCPs from eggs. Nevertheless, we used this in-line fat retention approach knowing that a certain amount of coextracted lipid in the off-line extract could be tolerated by the GC-ECD system (Hopper, 1987). In addition to in-line lipid trapping, we collected the OCPs off-line on a commercial solid phase extraction cartridge using the same system reported by Maxwell et al. (1992), who used this method to effectively separate nitrobenzamide coccidiostats from chicken liver. Our use of silica gel in-line for this purpose resulted in higher OCP recoveries and slightly cleaner chromatograms after SFE.

In addition to some initial SFE collection problems, we obtained highly variable GC-ECD responses with the OCP standards. A reduction of the injection port temperature from 250 to 220 °C resulted in less breakdown of DDT to DDE and of the endosulfans to an unidentified early-eluting compound. The use of an autoinjector and lowering the detector temperature from the 275–300 °C range to 260 °C resulted in an overall reduction of the instrument CVs for the OCPs from 10–22% to <5%. In addition, the GC chromatographic conditions were further optimized to give the best separation for the 16 OCPs. A typical chromatogram of the OCP standards is shown in Figure 2A, that of an unfortified egg extract in Figure 2B, and that of a fortified whole egg in Figure 2C. Parts B and C of Figure 2 show the only peaks not completely resolved were dieldrin and *p,p′*-DDE (peaks 9 and 10). In the egg-containing extracts, Figure 2B,C, there is a noninterfering peak at 17.7 min. There is also one peak at ~32 min that occurs occasionally in egg samples which could interfere with later eluting OCPs. When it was necessary to remove this peak, the *additional Florisil cleanup* step described under Experimental Procedures was employed. After treatment, the extract maintained recoveries of 85–90% for all of the OCPs. During the development of this cleanup procedure, Florisil was found to be the source of the ECD peak at 17.7 min in Figure 2B. It was removed by prewashing the Florisil with acetone and subsequent oven-drying.

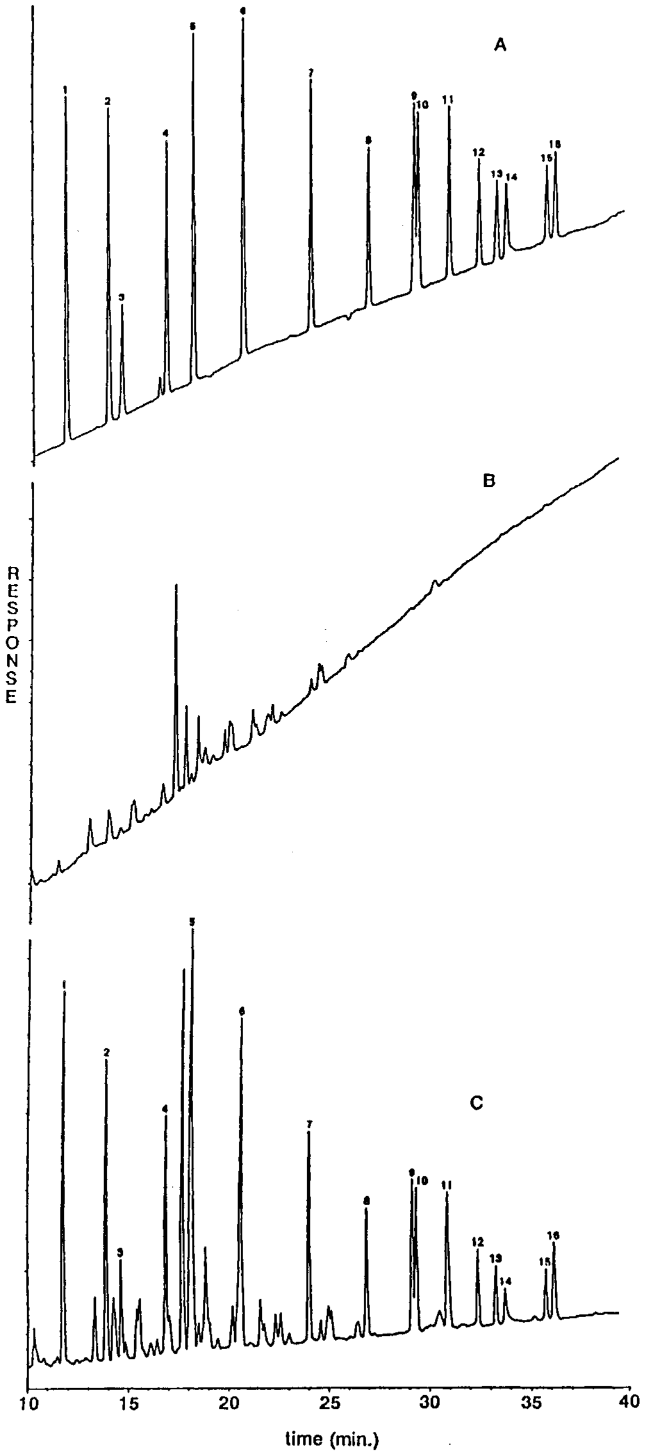

Figure 2. GC-ECD chromatograms of (A) mixed pesticide standard, (B) unfortified whole egg control, and (C) fortified whole egg sample. Pesticide elution is listed in Table 1.

SFE recovery studies ($n = 5$) were performed on eggs fortified at the 0.05, 0.10, and 0.20 ppm levels. For the latter two concentrations, the recoveries ranged from 76.0 to 102.9% and from 79.1 to 101.8%, respectively, with CVs varying from 1.9 to 11.1% at both levels. No significant ($p < 0.05$) differences were found among recoveries at the different concentrations, and, as an example, we are showing the individual OCP recoveries for the lowest concentration, 0.05 ppm (Table 1) in the order of their GC elution. At 0.05 ppm, the recoveries for the individual OCPs varied from 81.8 to 108.3%, with CVs ranging from 2.9 to 9.8%. Over 90% recoveries were obtained for many of the more polar OCPs, that is, heptachlor epoxide, endrin aldehyde, endosulfans I and II, and endosulfan sulfate. We obtained considerably higher recoveries for the same OCPs than those reported by Wigfield et al. (1996), who used both unmodified SC-CO_2 and methanol-modified CO_2, off-line cryogenic glass bead trapping, and post-extraction C_{18} and Florisil SPE cleanup in their SFE investigation of eggs fortified with 22 OCPs. Whereas the use of methanol modifier in the Wigfield study increased the recoveries of several OCPs and decreased it for others, it introduced a matrix interference effect. They eliminated this interference by conducting the extractions in pure CO_2, which gave recoveries of 50–112% for the 22 OCPs at the 0.05 ppm spiking level. Of these, α-HCB (α-BHC), lindane (γ-BHC), and α-endosulfan (endosulfan I) were <65%. In our study, we obtained higher recoveries for many of the same OCPs, including recoveries of 92, 103, and 96% for the same three OCPs indicated above. It is not precisely clear what factor(s) caused the lower recoveries in the Wigfield investigations. Initially, we carried out extractions at pressures <680 atm and also found that the OCPs were not completely extracted. Subsequent trials were carried out at the higher pressure, where

Table 1. Recoveries of OCPs from Fortified Whole Egg[a]

pesticide	recoveries, mean % (CV)	
	SFE method	AOAC/FDA method
α-BHC	92.0 (8.1)	94.0 (2.5)
γ-BHC	102.9 (2.7)	92.5 (1.7)
β-BHC	92.1 (10.7)	89.4 (1.7)
δ-BHC	100.6 (2.6)	95.1 (9.5)
heptachlor	81.9 (11.0)	27.0 (7.4)
aldrin	84.7 (9.9)	87.7 (3.8)
heptachlor epoxide	96.1 (2.7)	82.3 (1.4)
endosulfan I	96.1 (3.5)	86.9 (5.6)
dieldrin	91.0 (7.0)	75.5 (5.4)
p,p′-DDE	85.1 (10.4)	69.5 (3.9)
endrin	105.2 (4.6)	85.7 (2.9)
endosulfan II	98.6 (3.0)	35.0 (10.7)
p,p′-DDD	96.3 (3.0)	68.2 (4.5)
endrin aldehyde	97.9 (4.0)	3.0 (96.3)
endosulfan sulfate	91.8 (6.6)	11.1 (29.1)
p,p′-DDT	108.3 (7.0)	113.5 (1.9)

[a] Fortified at the 0.05 ppm level ($n = 5$).

Table 2. Isolation of Incurred Endosulfan I in Eggs by SFE and the AOAC/FDA Solvent Extraction

collection day	SFE (ppb)	AOAC/FDA (ppb)
0 (control)	0.00	0.00
1	0.00	0.00
2	1.96	1.13
3	18.20	16.94
4	53.88	42.36
5	7.65	7.82
6	5.05	5.97

increased recoveries were obtained. Although many variables affect extraction efficiency, we attribute at least part of the difference in our results to the higher extraction efficiency obtained by the use of the increased SC-CO_2 fluid density, pressure 680 atm at 40 °C, compared to 250 atm at 60 °C.

To provide more assurance of the effectiveness of SFE, the same fortified samples were analyzed according to the AOAC/FDA solvent extraction method (AOAC, 1996; FDA, 1994). The results are also shown in Table 1. Eleven of the 16 OCP recoveries were significantly lower than those obtained by using the SFE method; of these, four had recoveries <40%.

A significant difference in extractability between fortified and incurred samples can occur due to differences in binding characteristics and other factors. Therefore, to determine whether such differences occurred, a comparison study was carried out using SFE/SC-CO_2. Egg-laying chickens were fed two consecutive single daily doses of endosulfan I. This OCP is in widespread use as a replacement for the more toxic and persistent OCPs such as DDT and dieldrin (Lee et al., 1995) and is sometimes found in eggs (Schenck, 1996). To compare incurred with control samples, eggs were also collected from undosed control chickens. As shown in Table 2, most of the eggs contained <0.05 ppm (50 ppb) of endosulfan I. Here, too, the SFE values, with a few exceptions, were consistently higher than those obtained by using the AOAC/FDA method and were in good quantitative agreement with the results found in the fortified−recovery study. The CVs and repeatabilities for SFE and the solvent extraction procedure for endosulfan I were 9.6% (1.5 ppb) and 2.8% (0.4 ppb), respectively. The higher values obtained for the SFE method can most likely be attributed to the use of a different sample size, 1 versus 25 g, where lower levels of coextractives reduce background interference. Insofar as the translocation of endosulfan I into the egg, the maximum amount was detected on the third day after dosing followed by an apparent slow decline.

CONCLUSION

Our findings show that 16 common OCPs can be effectively recovered from eggs, and clean sample extracts can be obtained using the SFE conditions employed in this study. This SFE sample preparation method with its use of silica gel in the extraction vessel and off-line Florisil SPE trapping requires minimal sample manipulation and only a small amount of organic solvent (8 mL of acetone/hexane) per sample. This attribute compares favorably to the >1 L/sample used in the AOAC/FDA solvent extraction method (AOAC, 1996; FDA, 1994) and 70 mL/sample solvent reported for a conventional solid phase extraction method (Schenck et al., 1994). Eight to 10 samples per day can be readily analyzed with this SFE method. Additionally, the SFE method significantly reduces the cost of solvent purchase and disposal and reduces the safety hazards due to exposure to potentially flammable and toxic solvents.

LITERATURE CITED

Agriculture and Agri-Food Canada. *The Determination of Organochlorine, Polychlorinated Biphenyls and Toxaphene Residues in Eggs and Egg Products by GLC/ECD*; Report LSD No. P-RE-00L-88-EGG; 1993.

Alley, E. G.; Lu, G. Analysis of polychlorinated biphenyls in fatty biological matrices by on-line supercritical fluid extraction and supercritical fluid cleanup. *J. AOAC Int.* **1995**, *78*, 1051−1054.

Anonymous. High pesticide levels persist worldwide. *Res. Dev. Mag.* **1995**, *37*, 11.

AOAC. Organochlorine and organophosphorus pesticides. In *Official Methods of Analysis*, 16th ed.; AOAC International: Gaithersburg, MD, 1996; Section 10.1.01.

Ashraf-Khorassani, M.; Taylor, L. T.; Schweighardt, F. K. Development of a method for extraction of organochlorine pesticides from rendered chicken fat via supercritical fluoroform. *J. Agric. Food Chem.* **1996**, *44*, 3540−3547.

Colborn, T.; Dumanoski, D.; Myers, J. P. *Our Stolen Future*; Penguin Books USA: New York, 1996; pp 189−209.

Eggs for Industry. Eggs: the all natural ingredient. *Food Technol.* **1997**, *51*, 33−36.

EPA. Pollution Prevention Strategy. *Fed. Regist.* **1991**, *56*, 7849−7864.

FDA. Multiresidue determination of chlorinated pesticide and industrial chemical residues. In *Pesticide Analytical Manual*, 3rd ed.; Food and Drug Administration: Washington, DC, 1994; Vol. I, Section 304E4.

Federal Register. Title 3. Executive Order 12856, Federal Compliance with Right-to-Know Laws and Pollution Prevention Requirements. **1993**, *58* (Aug 6, 150), 41981−41987.

France, J. E.; King, J. W.; Snyder, J. M. Supercritical fluid-based cleanup technique for the separation of organochlorine pesticides from fats. *J. Agric. Food Chem.* **1991**, *39*, 1871−1874.

Hopper, M. L. Modified inlet for injecting a bonded phase widebore fused silica capillary column with chlorinated pesticides in lipid extract. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1987**, *10*, 620−622.

Hooper, M. L. Extraction and cleanup of organochlorine and organophosphorous pesticide residues in fats by supercritical fluid techniques. *J. AOAC Int.* **1997**, *80*, 639−649.

Hooper, M. L. An automated SFE and in-line cleanup system for the analysis of pesticide residues in fatty matrices. Presented at the 8th International Symposium on SFC/SFE, St. Louis, MO, 1998; C-09.

Janda, V.; Steenbeke, G.; Sandra, P. Supercritical fluid extraction of s-triazine herbicides from sediment. *J. Chromatogr.* **1989**, *479*, 200−205.

King, J. W.; Snyder, J. M.; Taylor, S. L.; Orton, W. L. Optimisation of experimental conditions for the SC−CO_2 extraction of pesticide residues from grains. *J. AOAC Int.* **1993**, *76*, 857−864.

Lee, N.; Skerritt, J. H.; McAdam, D. P. Hapten synthesis and development of ELISAs for detection of endosulfan in water and soil. *J. Agric. Food Chem.* **1995**, *43*, 1730−1739.

Maxwell, R. J.; Parks, O. W.; Piotrowski, E. G. Improved SFE recovery of trace analytes from liver using an integral micrometering valve-SPE column holder assembly. *J. High Resolut. Chromatogr.* **1992**, *15*, 807−811.

Schenck, F. L.; Wagner, R.; Hennessy, M. K.; Okrasinski, J. L. Screening procedure for organochlorine and organophosphorus pesticide residues in eggs using solid-phase extraction cleanup and gas chromatographic detection. *J. AOAC Int.* **1994**, *77*, 1036−1040.

Senedecor, G. W.; Cochran, W. G. *Statistical Analysis*, 6th ed.; Iowa State University Press: Ames, IA, 1979.

Snyder, J. M.; King, J. W.; Rowe, L. D.; Woerner, J. A. Supercritical fluid extraction of poultry tissues containing incurred pesticide residues. *J. AOAC Int.* **1993**, *76*, 888−892.

Statistical Analysis System Institute, Inc. In *SAS User's Guide: Statistics*; Cary, NC, 1985.

Thomas, P. T. Pesticide-induced immunotoxicity—are the Great Lakes residents at risk. *Environ. Health Perspect.* **1995**, *103* (Suppl. 9), 55−61.

Wigfield, Y. Y.; Selwyn, J.; Khan, S.; McDowell, R. Comparison of supercritical fluid extraction and solvent extraction of 22 organochlorine pesticides from eggs. *Chemosphere* **1996**, *32*, 841−847.

Youden, W. J.; Steiner, E. H. *Statistical Manual of the Association of Official Analytical Chemists*; AOAC International: Arlington, VA, 1975.

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