Supercritical Fluid-Based Cleanup Technique for the Separation of Organochlorine Pesticides from Fats

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A supercritical fluid cleanup technique for the separation of organochlorine pesticides from fats has been developed. The technique uses either an alumina column or a silica column with a supercritical mobile phase of carbon dioxide or methanol/carbon dioxide (2 mol % methanol), respectively. The lipid matrices were chicken fat with incurred residues of heptachlor epoxide, dieldrin, and endrin at low parts per million levels and lard spiked with lindane, heptachlor, hetachlor epoxide, dieldrin, endrin, and *o,p'*-DDT at low parts per million levels. Recoveries (93–111%) and precision (% RSD ≤ 8.5) obtained with the supercritical fluid technique compare favorably with those obtained by conventional column cleanup methodology.

In recent years, interest in the use of supercritical fluid extraction (SFE) as an analytical technique has increased considerably (Hawthorne, 1990). An interesting application of this technology is in the field of pesticide residue analyses. For instance, McNally and Wheeler (1988a,b) applied SFE to the analysis of sulfonylureas in soil, plant materials, and cell culture media. Engelhardt and Gross (1988) reported the analysis of lindane, aldrin, and *p,p'*-DDT in a spiked soil sample using SFE combined with supercritical fluid chromatography (SFC). Basic solubility studies of pesticides in supercritical carbon dioxide have been conducted by Schafer and Baumann (1988, 1989). Recently, Lopez-Avila et al. (1989) reported the SFE of 17 organochlorine pesticides and 25 organophosphorus pesticides from sand using carbon dioxide or acetone-modified carbon dioxide.

For the studies cited above, the sample matrices are devoid of coextractives that interfere in the subsequent analyses. Such is not the situation for sample matrices containing high lipid levels. Supercritical carbon dioxide readily solubilizes fatty acids, diglycerides, and triglycerides as well as minor lipid components such as sterols. Despite this fact, SFE has been used successfully in pesticide residue analyses of fatty samples. Fish tissue (Nam et al., 1988), lard (King, 1989), and chicken tissues (Snyder et al., 1990) containing organochlorine pesticides have been quantitatively extracted using supercritical carbon dioxide. However, since lipid components were coextracted, procedures to clean up the extracts were required prior to determination of the residues by gas chromatography. The above researchers employed conventional cleanup methods that utilize columns of silica gel or alumina on which the pesticides are selectively eluted with organic solvents.

The replacement of cleanup techniques that require organic solvents with ones that utilize carbon dioxide would extend the beneficial aspects of SFE to the cleanup steps. Organic solvent use and its attendant waste disposal problems would be minimized not only in the extraction step but also in the cleanup procedures that follow. A supercritical fluid cleanup technique also has the potential to perform both SFE and cleanup in one combined step. Our ultimate goal for such a supercritical fluid cleanup technique would be to couple it directly to SFE, thereby providing an integrated extraction/cleanup technique for pesticide residues in animal tissues. The described research represents our initial studies in the development of a cleanup technique that uses supercritical carbon dioxide with a preparative cleanup column to separate organochlorine pesticides from fat.

EXPERIMENTAL PROCEDURES

Sample Preparation. Incurred poultry fat samples were acquired from a prior study that examined the SFE of incurred residues from chicken tissues (Snyder et al., 1990). The fat samples contained heptachlor epoxide, dieldrin, and endrin at low parts per million levels and were obtained from adipose tissue using SFE. Commercial lard was obtained from a local abattoir. Lard samples were spiked with lindane, heptachlor, heptachlor epoxide, dieldrin, endrin, and *o,p'*-DDT in the range 0.5–2 ppm. Individual isooctane solutions of the pesticides (Supelco, Bellefonte, PA) were pipetted into lard heated above its melting point and then mixed well.

Apparatus. A supercritical fluid chromatograph (Hewlett-Packard 1082B, Avondale, PA) was modified to allow operation as a preparative chromatograph from which fractions could be easily collected. The alterations involved redirecting the exit line from the chromatographic column to bypass the UV detector. The exit line was connected directly to a restrictor that was vented into a 12-mL vial holding 5 mL of hexane for fraction collection. A fused silica capillary (Polymicro Technologies, Phoenix, AZ) of the dimensions 10 cm × 0.375 mm o.d. × 0.040 mm i.d. was used as the restrictor. It was attached to the exit line by a low dead volume fitting. Carbon dioxide was supplied to the chromatograph by a cylinder equipped with a dip tube. Flow rate was maintained at 1.0 mL (liquid)/min.

The collected pesticide fraction was analyzed with a gas chromatograph (Hewlett-Packard Model 5890) equipped with an electron capture detector and an automatic sample injector providing a 2-μL splitless injection. Peaks were quantified with an integrator (Hewlett-Packard Model 3396A). For the incurred fat samples, the column was a 2 m × 4 mm i.d. glass column with a mixed stationary phase (GP 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport, Supelco). Oven temperature was held isothermally at 200 °C, injector at 220 °C, and detector at 350 °C. Helium carrier gas flow rate was 40 mL/min. For the spiked lard samples, a 30 m × 0.32 mm i.d. fused silica capillary column (DB-5, J&W Scientific, Folsom, CA) was used with temperature programming. Temperature was held at 100 °C for 1 min, ramped to 190 °C at 10 °C/min and then ramped to 250 °C at 3 °C/min and held at 250 °C for 5 min.

Carbon Dioxide Purity Evaluation. Cylinders of carbon dioxide from various sources and lots were analyzed for desired purity limits for use with ECD. Before sampling, the valve assembly on each cylinder was rapidly opened and closed

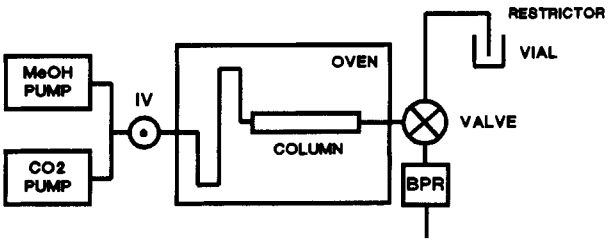

Figure 1. Schematic diagram of supercritical fluid cleanup system with column regeneration option (BPR, back-pressure regulator; IV, injection valve).

("burped") before the connecting tubing was attached. CO_2 was bubbled through hexane (3–6 mL) suitable for residue analysis. Depressurization and flow control was achieved with a 40–50 μm i.d. fused silica capillary of about 20 cm in length. A standard cylinder fitting was attached by means of a tee to a pressure gauge and the capillary restrictor. The capillary was held in place with a graphite/vespel ferrule (Alltech Associates, Deerfield, IL). Flow rates were measured by connecting a soap bubble meter to the restrictor. Prior to collection, the depressurization assembly was purged with CO_2 for at least 30 min. Collection time was adjusted to allow 10 L (at NTP) of CO_2 to bubble through the hexane. Once collection was completed, the hexane sample was treated in a manner identical to that described below for the pesticide fractions.

Cleanup Procedures. The supercritical fluid-based cleanup procedure was compared to a conventional cleanup method taken from the *Revised Basic Chemistry Laboratory Guidebook* (1987) of the U.S. Department of Agriculture, Food Safety and Inspection Service. The conventional method involves separating the chlorinated hydrocarbons from the fat by elution with hexane or petroleum ether in a glass column filled with partially deactivated alumina. Two slightly different procedures using supercritical fluid mobile phases were investigated. The first approach used a stainless steel column of 7-cm length, 4.6 mm i.d., and 6.4 mm o.d. Precautions should be taken to ensure that the tubing and fittings are rated to withstand the pressures at the desired operating conditions. The alumina (neutral Brockman I) was deactivated with 5 wt % deionized water, and 1.4 g of the alumina was hand packed into the column. As is done in the conventional method, the alumina is used once and then discarded. Before sample injection, the alumina column was conditioned in the carbon dioxide flow at the same experimental parameters used during elution.

The second experimental approach utilized a column of the same size containing 0.5 g of silica (PN 51900, Millipore, Waters Division, Milford, MA). After the pesticide fraction was collected, the retained lipid fraction was eluted with 4–6 mol % methanol in carbon dioxide with all other conditions being the same as reported in Table II. A valve was placed downstream of the cleanup column to divert the fat-laden effluent through the back-pressure regulator of the SFC unit rather than through the fused silica restrictor (Figure 1). After this step, the column was reconditioned by flowing unmodified carbon dioxide through the column for 20 min. In this manner the silica column was used for the duration of the study. With both approaches an injection valve with a sample loop of 50 μm was utilized. Neat fat or lard was injected as a liquid after warming on a hot plate.

For all of the experiments, the carbon dioxide mobile phase was depressurized directly into hexane. The hexane was then evaporated to 0.5 mL using dry nitrogen. For the chicken fat analyses, 0.5 mL of a solution of aldrin (100 ng/mL) in 2,2,4-trimethylpentane was added as an internal standard. This mixture was then pipetted into crimp-top vials for the GC/ECD analysis. For the spiked lard analyses, heptachlor epoxide, one of the pesticide spikes, was used as the internal standard.

RESULTS AND DISCUSSION

Purity of Carbon Dioxide. As with conventional solvents used in pesticide residue analysis, the purity of the carbon dioxide must be examined to ensure that it is adequate for trace analysis. Several gas cylinders from various sources were examined for ECD residues. Figure 2 displays a few of the chromatograms generated in the evaluation of CO_2 purity. The cleanest CO_2 in our survey came from a "SFC–SFE" CO_2 cylinder (Figure 2a) and a "food grade" CO_2 cylinder (Figure 2b). An example of a seriously contaminated gas is displayed in Figure 2c. In this case, the carbon dioxide sample was obtained from a "welding grade" CO_2 cylinder. Another cylinder identified as "SFC" CO_2 also exhibited an unacceptable level of contamination as shown in Figure 2d. These results clearly illustrate the need to evaluate the CO_2 purity of each gas cylinder. Capillary GC with electron ionization mass spectrometry tentatively identified the major contaminants in the welding grade CO_2 as an oligomeric mixture of perfluorochlorocarbons. Similar residue problems were cited by Nielen et al. (1989). In their study, food grade carbon dioxide was found to be acceptable. With proper pretesting precautions, carbon dioxide with adequate purity was identified and utilized.

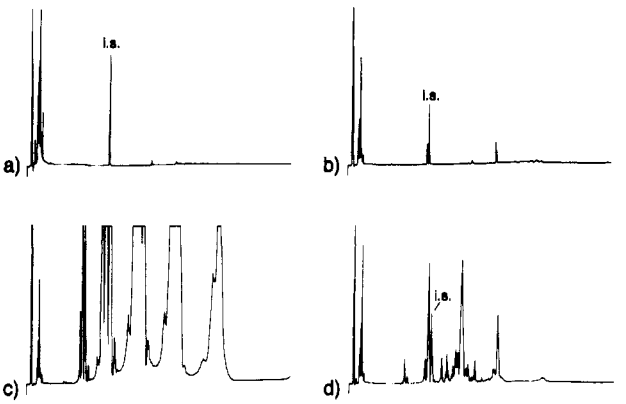

Figure 2. Capillary GC/ECD chromatograms of various carbon dioxide cylinders specified as grades: (a) SFE–SFC; (b) food; (c) welding; (d) SFC.

Table I. Comparison of Cleanup Techniques on Chicken Fat[a]

	supercritical fluid (n = 8)		conventional (n = 9)	
pesticide	mean	% RSD	mean	% RSD
heptachlor epoxide	0.84	8.5	0.84	8.6
dieldrin	3.00	5.9	3.13	7.1
endrin	2.48	3.5	2.60	7.4

[a] Statistical means are in milligrams of pesticide per kilogram of fat; % RSD, percent relative standard deviation due to cleanup technique and GC/ECD analysis. Conditions for supercritical fluid cleanup: column, 1.4 g of alumina; column temperature, 40 °C; column pressure, 190–270 bar; flow rate, 1.0 mL (liquid CO_2)/min; collection time, 20 min; collection vial, 12-mL vial with 5 mL of hexane at start; modifier addition, none; amount of coeluted lipid, <1 mg (not measured in all runs).

Organochlorine Pesticide Determinations. Results from repetitive analyses of the same chicken fat by the supercritical fluid cleanup technique and the conventional method are listed in Table I. On the basis of the f-test and the t-test, precisions and recoveries for the two cleanup techniques are not statistically different at the 95% confidence limit. On the basis of these results, the supercritical fluid technique is judged to be equivalent to the standard method.

An examination of representative chromatograms of samples cleaned up by the two techniques further confirms that the supercritical fluid cleanup technique yields equivalent results. The packed column GC/ECD chromatograms A and D in Figure 3 are of SFE fat samples treated by the supercritical fluid cleanup technique and

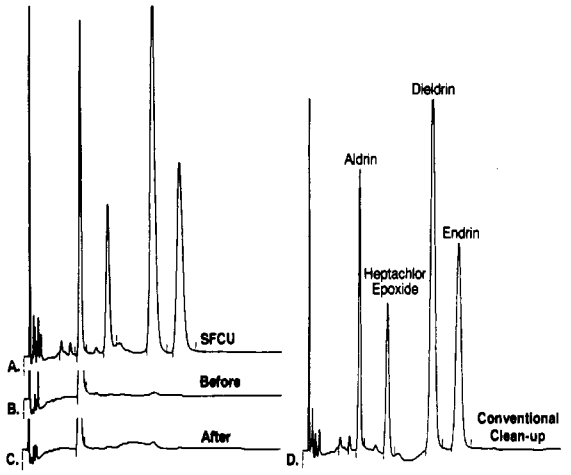

Figure 3. Comparison of packed column GC/ECD chromatograms of incurred pesticide residues in SFE fat from poultry adipose tissue. (A) Supercritical fluid cleanup (SFCU); (B) blank CO_2 collection (20 min) prior to fat injection; (C) fraction collected immediately following sample, 10-min collection time; (D) conventional cleanup methodology.

the conventional cleanup, respectively. Prior to the neat injection of fat into the supercritical fluid cleanup column, a CO_2 blank was collected under the same conditions and length of time. No substantial contribution to the ECD signal is exhibited in the region where the pesticides elute (Figure 3B). Only the internal standard, aldrin, was observed. It is apparent that no interfering contaminants were present, indicating that the carbon dioxide as well as the SFE equipment was sufficiently clean. A followup fraction was also collected (Figure 3C). The lack of chromatographic peaks shows that the initial 20-min collection was sufficient for total pesticide recovery.

A second approach to performing supercritical fluid cleanup utilized a silica column that was reused repetitively. After the pesticide and lipid separations were performed, the retained lipid was eluted from the column by using methanol-modified carbon dioxide as the mobile phase. This approach eliminated two problems associated with the alumina column. One problem was the continual opening and closing of the fittings to dispose of the used alumina. The fittings would eventually wear out with extended use and then leak when under pressure. Second, the need to predry the alumina column under carbon dioxide flow was avoided. Water is used to deactivate the alumina [5 wt % water added per *Revised Basic Chemistry Laboratory Guidebook* (1987)]. Since water has a finite solubility in supercritical carbon dioxide (Evelein et al., 1976), it is taken up by the carbon dioxide. Depressurization of the carbon dioxide through the restrictor causes the water to precipitate and freeze. Ice would form in the restrictor, producing inconsistent flows and uncontrolled increases in column pressure.

The schematic diagram in Figure 1 illustrates the enhanced cleanup system in which a valve is placed downstream of the cleanup column. The valve allows the lipid-laden effluent to be diverted through a back-pressure regulator (BPR). This arrangement avoids any fouling of the restrictor with lipid material. Since the column is regenerated in situ, there is also no need for repetitively opening and closing the column fittings. Moreover, methanol is used as the deactivating agent for the sorbent column rather than water. The use of methanol solved the flow problems that were experienced with water deactivation of the sorbent column.

Early in the work on spiked lard with the enhanced system it was apparent that recoveries of dieldrin and

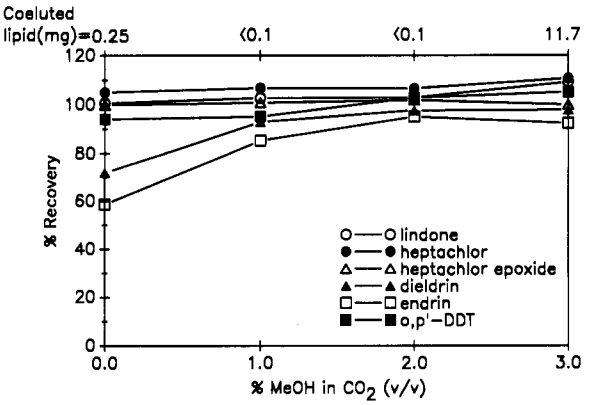

Figure 4. Effect of methanol addition on the supercritical fluid cleanup of spiked lard. Conditions: 0.5-g silica column; 40 °C; 1.0 mL (liquid)/min; 10-min collection time; 200-atm column pressure; 0.5 h of column conditioning prior to the introduction of each sample. Percent recoveries are calculated by ratioing supercritical fluid cleanup recoveries to conventional cleanup recoveries.

Table II. Comparison of Recoveries and Precisions of Recoveries for Organochlorine Pesticides from Spiked Lard[a]

	supercritical fluid[b] (n = 5)		conventional (n = 6)	
pesticide	mean	SD	mean	SD
lindane	0.62	0.02	0.59	0.01
heptachlor	0.78	0.05	0.70	0.02
dieldrin	0.58	0.01	0.62	0.03
endrin	0.83	0.02	0.89	0.04
o,p'-DDT	1.66	0.05	1.70	0.09

[a] Statistical means are in milligrams of pesticide per kilogram of lard; heptachlor epoxide used as an internal standard. [b] Conditions for supercritical fluid cleanup: column, 0.5 g of silica; column temperature, 50 °C; column pressure, 190–270 bar; flow rate, 1.0 mL (liquid CO_2)/min; collection time, 20 min; collection vial, 12-mL vial with 5 mL of hexane at start; modifier addition, 2.5% (v/v) methanol; amount of coeluted lipid, 2.1 mg.

endrin were unsatisfactory for elution times of 10–20 min. Therefore, methanol was added in small amounts as a modifier to improve recoveries. Results from this study are shown in Figure 4. The graph shows the effect that the amount of methanol in the CO_2 mobile phase has on pesticide recovery. As observed in Figure 4, recoveries for dieldrin and endrin were poor when no methanol was added. The addition of small amounts of methanol improved the recoveries of dieldrin and endrin but did not affect recoveries for the other organochlorine pesticides. At 3.0% methanol in CO_2, the large amount of coeluting lipid indicates that the mobile phase has an elutropic strength that is too high to achieve the desired lipid/pesticide separation.

The mean recoveries and standard deviations of pesticide residues in a spiked lard as determined by supercritical fluid cleanup and conventional approaches are compared in Table II. For the pesticides investigated, the means of the two methods agree fairly well. At the 99% confidence limit, only the heptachlor values are considered to be significantly different, and this only by a small margin. The ranges encompassed by the standard deviations are comparable, indicating that the supercritical fluid cleanup techniques has a precision similar to that of the conventional cleanup method. Capillary GC/ECD chromatograms of spiked lard samples cleaned up according to the two procedures are compared in Figure 5. With an adequately clean source of CO_2, no interfering peaks are noticed with the supercritical fluid cleanup technique.

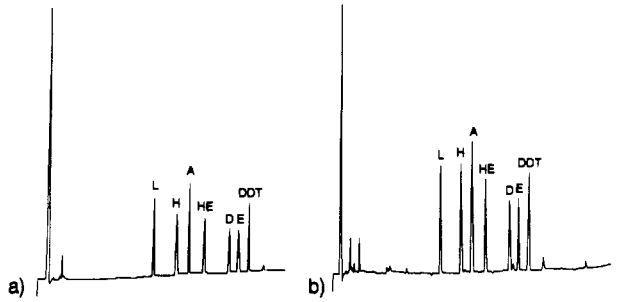

Figure 5. Capillary GC/ECD chromatograms of spiked lard samples from (a) supercritical fluid and (b) conventional cleanups (L, lindane; H, heptachlor; HE, heptachlor epoxide; D, dieldrin; E, endrin; DDT, *o,p'*-DDT).

The amount of coeluted lipid that can be tolerated by capillary GC/ECD has been experimentally determined (Hopper, 1987). From that information, a tolerance limit of approximately 3 mg/mL can be calculated for our analyses by correcting for differences in sample injection size and column dimensions. Both the alumina column and silica column cleanup techniques using supercritical fluids have been shown to be capable of performing separations of organochlorine pesticides from fats that achieve those limits. Furthermore, the recoveries for the pesticide residues are comparable to those achieved with the conventional organic solvent based column cleanup method. Future research will focus on integrating supercritical fluid cleanup techniques directly with SFE for pesticide residue analysis of fatty samples. Although conclusive proof will only be achieved through experimental verification, it is expected that all chlorinated hydrocarbon pesticides and fats that have been successfully cleaned up with the conventional column methodology should be amenable to the supercritical fluid technique.

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LITERATURE CITED

Engelhardt, H.; Gross, A. Extraction of Pesticides from Soil with Supercritical CO_2. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1988**, *11*, 726.

Evelein, K. A.; Moore, R. G.; Heidemann, R. A. Correlation of the Phase Behavior in the Systems Hydrogen Sulfide-Water and Carbon Dioxide-Water. *Ind. Eng. Chem., Process Des. Dev.* **1976**, *15*, 423–428.

Hawthorne, S. B. Analytical-Scale Supercritical Fluid Extraction. *Anal. Chem.* **1990**, *62*, 633A–642A.

Hopper, M. L. Modified Inlet for Injecting a Bonded Phase Wide-Bore Fused Silica Capillary Column with Chlorinated Pesticides in a Lipid Extract. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1987**, *10*, 620–622.

King, J. W. Fundamentals and Applications of Supercritical Fluid Extraction in Chromatographic Science. *J. Chromatogr. Sci.* **1989**, *27*, 355–364.

Lopez-Avila, V.; Beckert, W. F.; Billets, S. The Why and How of Supercritical Fluid Extraction and Its Application to Environmental Analysis. In *Proceedings of the 5th Annual Waste Testing and Quality Assurance Symposium*; U.S. EPA: Washington, DC, 1989; Vol. II, pp II-73–II-86.

McNally, M. E. P.; Wheeler, J. R. Supercritical Fluid Extraction Coupled with Supercritical Fluid Chromatography for the Separation of Sulfonylurea Herbicides and Their Metabolites from Complex Matrices. *J. Chromatogr.* **1988a**, *435*, 63–71.

McNally, M. E. P.; Wheeler, J. R. Increasing Extraction Efficiency in Supercritical Fluid Extraction from Complex Matrices: Predicting Extraction Efficiency of Diuron and Linuron in Supercritical Fluid Extraction Using Supercritical Fluid Chromatographic Retention. *J. Chromatogr.* **1988b**, *447*, 53–63.

Nam, K. S.; Kapila, S.; Pieczonka, G.; Clevenger, T. E.; Yanders, A. F.; Viswanath, D. S.; Mallu, B. Supercritical Extraction of Hazardous Chemicals from Fish Tissue. In *Proceedings of the International Symposium on Supercritical Fluids*; Perrut, M., Ed.; Institute National Polytechnique de Lorraine: Lorraine, France, 1988; Tome 2, pp 743–750.

Nielen, M. F. W.; Sanderson, J. T.; Frei, R. W.; Brinkman, U. A. Th. On-line System for Supercritical Fluid Extraction and Capillary Gas Chromatography with Electron-Capture Detection. *J. Chromatogr.* **1989**, *474*, 388–395.

Revised Basic Chemistry Laboratory Guidebook. Section 5.002, Micro Alumina Screening Analysis for Chlorinated Hydrocarbons and PCB Residues in Fatty Foods; U.S. Department of Agriculture, Food Safety and Inspection Service: Washington, DC, June 1987; pp 5–9.

Schafer, K.; Baumann, W. Solubility of Some Pesticides in Supercritical CO_2. *Fresenius' Z. Anal. Chem.* **1988**, *332*, 122–124.

Schafer, K.; Baumann, W. Supercritical Fluid Extraction of Pesticides. I. Extraction Properties of Selected Pesticides in CO_2. *Fresenius' Z. Anal. Chem.* **1989**, *332*, 884–889.

Smith, R. D.; Wright, B. W.; Yonker, C. R. Supercritical Fluid Chromatography: Current Status and Progress. *Anal. Chem.* **1988**, *60*, 1323A–1336A.

Snyder, J. M.; King, J. W.; Rowe, L. D.; Woerner, J. A. Analysis of Incurred Chlorinated Pesticide Residues from Supercritical Fluid Extracted Chicken Tissues. *J. Assoc. Off. Anal. Chem.* **1991**, submitted for publication.

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