Determination of Organochlorine and Organophosphorus Pesticide Residues in Eggs Using a Solid Phase Extraction Cleanup

Frank J. Schenck*

Southeast Regional Laboratory, U.S. Food and Drug Administration, 60 Eighth Street N.E., Atlanta, Georgia 30309

Dan J. Donoghue

Center for Veterinary Medicine, U.S. Food and Drug Administration, 8401 Muirkirk Road, Laurel, Maryland 20708

A multiresidue solid phase extraction (SPE) method for the isolation and subsequent gas chromatographic determination of nonpolar organochlorine and polar organophosphorus pesticide residues in eggs is described. The method uses an acetonitrile extraction followed by an SPE cleanup using graphitized carbon black and aminopropyl SPE columns. Organophosphorus pesticides are determined by gas chromatography with flame photometric detection. After further cleanup of the extract using Florisil SPE columns, organochlorine pesticides are determined by gas chromatography with electron capture detection. Studies were performed using eggs containing both fortified and incurred pesticide residues. The average recoveries were 86−108% for 8 fortified organochlorine pesticide residues and 61−149% for 28 fortified organophosphorus pesticide residues.

Keywords: *Eggs; organochlorine pesticides; organophosphorus pesticides; solid phase extraction*

INTRODUCTION

Recent studies have shown that if chickens are exposed to either polar or nonpolar residues, these residues can be detected in the egg yolks long after the residue is eliminated from the rest of the chicken (Donoghue et al., 1995, 1996, 1997). Yolks develop in the ovary over a period of days to weeks, and yolks that are days to weeks from ovulation will incorporate and store both polar and nonpolar residues.

The U.S. Food and Drug Administration (FDA) routinely analyzes eggs for organochlorine pesticide (OCP) residues and a limited number of organophosphorus pesticide (OPP) residues. The method used is found in both the AOAC's *Official Methods of Analysis* (Sawyer et al., 1999) and the FDA's *Pesticide Analytical Manual* (McMahon and Wagner, 1994). Whereas this method results in an efficient extraction and cleanup for the nonpolar OCPs and some relatively nonpolar OPPs, the polar OPPs cannot be recovered when using this method. Methods that can be used to recover the polar OPPs from eggs have been reported (Blaha and Jackson, 1985; Leoni et al., 1992). Both of these methods entail using an acetone extraction, followed by multiple methylene chloride partition steps. Further cleanup of the extract using either gel permeation chromatography or a charcoal Celite column is required.

The objective of this study was to develop a sensitive method that could be used for the quantitative determination of both nonpolar and polar pesticide residues in eggs. The method described below provides an excellent cleanup with a minimal number of steps and allows for the detection of a wide number of pesticide residues in eggs at trace levels.

* Author to whom corresponde should be addressed [telephone (404) 253-1200, ext. 5409; fax (404) 253-1208; e-mail fschenck@ora.fda.gov].

EXPERIMENTAL PROCEDURES

Reagents. All solvents were glass distilled, residue grade (EM Reagents).

Materials. The SPE columns used were as follows: Envi-Carb graphitized carbon black (GCB), 500 mg, 6.0 mL; aminopropyl, 500 mg, 3 mL; Florisil, 1.0 g, 6.0 mL (Supelco Corp., Bellefonte, PA). An N-Evap evaporator (Organomation Associates Inc., West Berlin, MA) was used for nitrogen evaporation. An explosion-proof Waring blender with a 1-L jar was used for extraction.

Standards. Pesticide reference standards were obtained from the U.S. Environmental Protection Agency (Laurel, MD). Individual stock standard solutions (0.2−1.0 mg/mL) were prepared in acetone. Stock standard solutions were admixed to yield spiking solutions, with standard concentrations of 0.5−50 ng/μL, in acetone, according to their respective sensitivities. Working standard solutions, in acetone (OPPs) or petroleum ether (OCPs), were prepared from spiking solutions.

Procedure. Yolks and whites of whole eggs were combined and blended at low speed until a homogeneous sample was obtained. A 10 g sample of egg was weighed into a blender cup. (For spike recoveries, a suitable mixed standard solution was added and allowed to stand for 5 min.) Acetonitrile (80 mL) was added to the blender cup. The sample was blended for 2 min at high speed and filtered with suction through a Büchner funnel fitted with filter paper into a vacuum flask. The filtrate was transferred to a 500-mL separatory funnel. Water (20 mL) and sodium chloride (8 g) were added, and the funnel was shaken for 1 min. After the phases were allowed to separate for 15 min, the lower aqueous phase was drained and discarded. Sodium sulfate (15 g) was added to the separatory funnel, and the funnel was shaken for 30 s. Exactly 40 mL of the dried acetonitrile extract was transferred to a 50-mL conical centrifuge tube and evaporated under a stream of nitrogen at 60 °C to ∼1 mL. Acetone (∼5 mL) was added to the centrifuge tube, and it was again evaporatee to ∼1 mL.

10.1021/jf000142c
Published on Web 12/01/2000

Table 1. Recoveries of Fortified OCPs from Eggs by the SPE Method

pesticide	recovery[a] at fortifn of 0.002 μg/g	0.01 μg/g	0.10 μg/g
p,p′-DDE	105.7 (15)	97.0 (6)	101.4 (2)
p,p′-DDT	107.9 (17)	97.1 (4)	106.9 (2)
dieldrin	102.4 (12)	98.4 (8)	103.4 (2)
heptachlor epoxide	99.4 (12)	98.0 (6)	98.9 (4)
lindane	92.3 (13)	92.6 (6)	85.8 (9)
trans-nonachlor	105.7 (16)	98.0 (6)	101.3 (3)

[a] $n = 3$; values in parentheses are coefficients of variation.

Table 2. Recoveries of Fortified OPPs from Eggs by the SPE Method

	fortifn (μg/g)	% recovery[a]	fortifn (μg/g)	% recovery[a]
acephate	0.04	95.3 (5)	0.80	64.7 (1)
azinphos-ethyl	0.03	98.9 (4)	0.60	103.7 (1)
azinphos-methyl	0.08	118.5 (12)	1.60	110.3 (1)
carbophenothion	0.01	78.8 (12)	0.20	93.5 (2)
α-chlorfenvinphos	0.01	88.9 (8)	0.20	94.1 (5)
chlorpyrifos	0.004	94.2 (7)	0.08	98.6 (5)
chlorpyrifos-methyl	0.005	91.3 (9)	0.09	90.4 (4)
cyanophos	0.003	114.5 (11)	0.07	94.0 (6)
diazinon	0.003	88.5 (11)	0.06	89.6 (4)
dicrotophos	0.02	95.9 (7)	0.30	103.9 (4)
dimethoate	0.005	148.5 (7)	0.10	107.5 (7)
ethion	0.006	88.9 (13)	0.10	98.2 (2)
fenamiphos	0.008	97.5 (8)	0.15	102.3 (2)
gardona	0.01	105.0 (7)	0.20	104.4 (5)
iodenofos	0.006	95.3 (6)	0.10	97.4 (5)
malathion	0.008	92.4 (8)	0.15	97.1 (4)
malathion oxygen analogue	0.006	92.3 (6)	0.15	102.9 (4)
methamidophos	0.003	83.2 (11)	0.07	61.0 (3)
methidathion	0.005	100.0 (8)	0.10	104.3 (4)
monocrotophos	0.005	109.5 (8)	0.10	84.3 (3)
omethoate	0.02	121.0 (9)	0.40	102.0 (1)
parathion	0.004	92.9 (6)	0.10	96.8 (3)
parathion oxygen analogue	0.007	96.9 (5)	0.10	103.2 (6)
phosmet	0.04	108.3 (6)	0.80	104.4 (7)
pirimiphos-methyl	0.004	86.5 (6)	0.07	94.6 (6)
pyrazophos	0.02	87.5 (10)	0.30	102.1(5)
quinalphos	0.006	91.6 (7)	0.10	98.8 (2)
ronnel (fenchlorphos)	0.005	87.0 (11)	0.10	87.9 (4)

[a] $n = 4$; values in parentheses are coefficients of variation.

Sodium sulfate (~1 cm) was added to a GCB SPE column, and an aminopropyl SPE column was attached beneath it. The tandem columns were conditioned with 5 mL of an acetone/toluene (3:1, v/v) mixture. The concentrated extract from the centrifuge tube was transferred to the GCB column. The centrifuge tube was rinsed twice with 2 mL of acetone/toluene (3:1, v/v), and the rinsings were transferred to the GCB column. Sufficient vacuum was applied to elute the column at a rate of 2−3 drops per second. The eluate was collected in a graduated, conical, glass, 15-mL centrifuge tube. The tandem columns were eluted two times with 5 mL of the acetone/toluene mixture, and the eluates were collected. The combined eluates were evaporated under a stream of nitrogen to <1 mL. Acetone (~10 mL) was added, and the eluate was evaporated to ~1 mL to make a solvent exchange to acetone. The volume was adjusted to 1.0 mL with acetone. A 3.0 μL aliquot of the eluate was injected into a gas chromatograph equipped with an FPD, for the determination of the OPPs.

A further cleanup of the extract was required for the low-level detection of the OCPs using the electron capture detector. Sodium sulfate (~2 cm) was added to a Florisil SPE column, and the column was washed with 5 mL of petroleum ether. A 15-mL graduated centrifuge tube was placed beneath the column. The acetone extract used for the determination of the organophosphorus pesticides was evaporated to dryness under a stream of nitrogen at 30 °C. Petroleum ether (~1.0 mL) was added to the centrifuge tube. The tube was vortex mixed, and the contents were transferred to the Florisil SPE column using a Pasteur pipet. The Florisil SPE column was allowed to elute by gravity flow. The centrifuge tube was rinsed two times with ~1.0 mL of petroleum ether, and the rinsings were transferred to the Florisil SPE column. The Florisil SPE column was eluted two times with 5 mL of a freshly prepared 2% (v/v) ethyl ether/petroleum ether mixture. The Florisil SPE column was eluted with a sufficient additional volume of 2% ethyl ether/petroleum ether to ensure that the total volume of the combined eluates collected in the centrifuge tube was ~15 mL. The eluate was evaporated under a stream of nitrogen and the final volume was adjusted to 1.0 mL with petroleum ether. A 3.0 μL aliquot was injected into a gas chromatograph equipped with an ECD, for the determination of the OCPs.

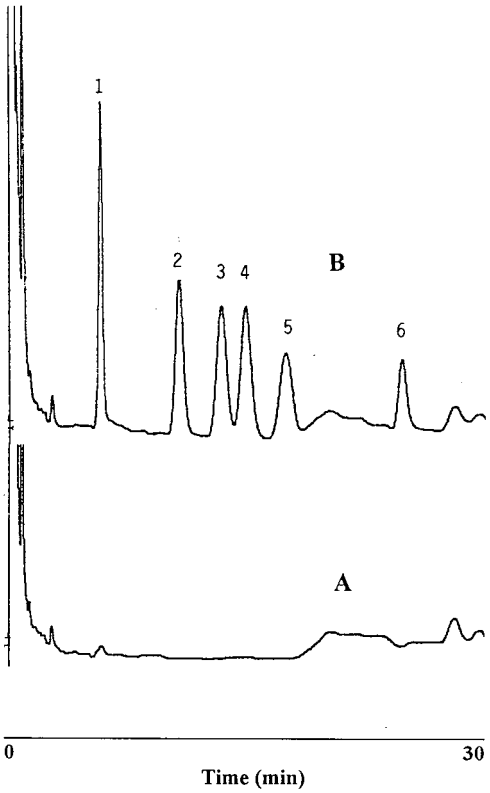

Figure 1. Chromatograms of control egg (A) and control egg fortified with 0.01 μg/g organochlorine pesticides (B). Peak identities are (1) lindane, (2) heptachlor epoxide, (3) *trans*-nonachlor, (4) *p,p′*-DDE, (5) dieldrin, and (6) *p,p′*-DDT.

Chromatography. Hewlett-Packard model 5890 gas chromatographs, equipped with ^{63}Ni electron capture detectors (ECD) or flame photometric detectors (FPD), were used. For GC/FPD, the analytical column was a 30 m, 0.53 mm i.d., 1.5-μm film thickness DB-5 wide-bore capillary column (J&W Scientific, Folsom, CA). The He carrier gas flow rate was 12 mL/min, and He makeup gas through the FPD was 10 mL/min. Detector temperature was 225 °C, and injection temperature was 220 °C using splitless injection into a 5 mm i.d. glass liner. The oven temperature program was 130 °C for 1 min and 6 °C/min until a final temperature of 225 °C was reached and held for 17 min. For GC/ECD, the analytical column was a 30 m, 0.53 mm i.d., 1.5-μm film thickness DB-225 wide-bore capillary column (J&W Scientific). The ultrahigh-purity helium carrier gas flow rate was 20 mL/min, and the argon/methane makeup gas flow rate was 10 mL/min. Detector temperature was 350 °C, and injection temperature was 250 °C using splitless injection into a 5 mm i.d. glass liner. The oven temperature program was 165 °C for 18 min and 10 °C/min until a final temperature of 190 °C was reached and held for 10 min.

Residue Quantitation. An appropriate working standard solution was chosen for each set of samples, such that the peak

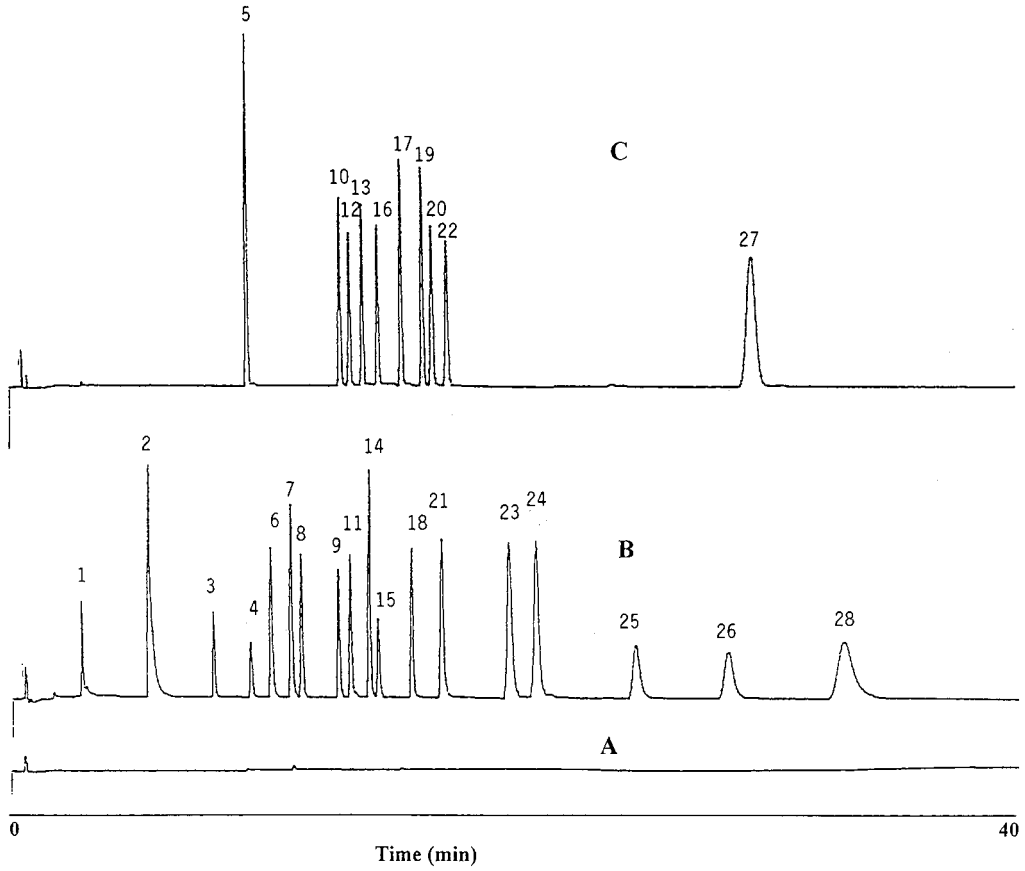

Figure 2. Chromatograms of control egg (A), control egg fortified (0.003−0.08 μg/g) with 18 organophosphorus pesticides (B), and control egg fortified (0.004−0.02 μg/g) with 10 organophosphorus pesticides (C). Peak identities are (1) methamidophos, (2) acephate, (3) monocrotophos (3), omethoate (4), dicrotophos (5), dimethoate (6), cyanophos (7), diazinon (8), chlorpyriphos-methyl (9), malathion oxygen analogue (10), ronnell (11), parathion oxygen analogue (12), pirimiphos-methyl (13), malathion (14), parathion (15), α-chlorfenvinphos (16), chlorpyriphos (17), quinalphos (18), methidathion (19), gardona (20), fenamiphos (21), iodenophos (22), ethion (23), carbophenothion (24), phosmet (25), azinphos-methyl (26), pyrazophos (27), and azinphos-ethyl (28).

height of the sample was within ±25% of the standard. Aliquots of standard and sample solution were injected alternately. A control sample extract was run along with each set of fortified or incurred samples.

Incurred Residue Study. Four hens (~1.5 kg body weight) were dosed orally with gelatin capsules at the following rates: chlorpyriphos-methyl, 9 mg/day for 9 days; dimethoate, 7.5 mg/day for 9 days; malathion, 60 mg/day for 3 days; and methoxychlor, 2 mg/day for 2 days. One egg was collected from each hen each day. Assuming a feed consumption of 120 g of feed a day for each hen, the corresponding (or equivalent) concentration of pesticide in the daily ration would be 83 μg/g for chlorpyriphos-methyl, 63 μg/g for dimethoate, 17 μg/g for methoxychlor, or 500 μg/g for malathion. These fairly high dosing rates were chosen because preliminary studies had shown that the transfer rate of pesticides into eggs was rather low, 0.1−1.0%.

Calculations. The equivalent sample weight injected is calculated as follows:

$$\frac{\text{mg of sample equivalent}}{\mu\text{L of final extract}} = 10 \times \frac{40}{80} \times 1$$

where 10 = g of sample analyzed, 40 = mL of dried acetonitrile extract taken through SPE cleanup, 80 = mL of acetonitrile in sample extract,and 1 = final volume of extract.

The amount of residue found is calculated using the chromatographic peak responses as follows:

$$\frac{\text{peak response of sample}}{\text{peak response of standard}} = \frac{\text{ng of standard injected}}{\text{mg of sample equivalent injected}}$$

RESULTS AND DISCUSSION

Recovery data were obtained by spiking eggs at two levels with OCPs and OPPs. The recoveries for 6 spiked OCPs ranged from 86 to 108% (see Table 1). The recoveries for 28 OPPs ranged from 61 to 149% (Table 2). None of the control eggs used for the spiking studies contained any detectable pesticide residues. The abnormally high recoveries observed for some of the OPPs were due to sample matrix enhancement (Erney et al., 1993). Matrix enhancement, a phenomenon commonly encountered in the gas chromatographic (GC) analysis of pesticides in foods, results in abnormally high recoveries of certain pesticides. Whereas thermal degradation/adsorption of certain pesticide residues takes place inside the injection inlet of the GC, the presence of coextracted sample matrix impurities may block these active sites, leading to an increase in the amount of analyte being transferred to the column.

The pesticide residues were extracted from the eggs using acetonitrile. The acetonitrile was separated from the water by salting out, dried with sodium sulfate, and concentrated. The concentrated extract was heavily pigmented, containing many sample matrix coextractants. The extract was cleaned up using tandem graphitized carbon black (GCB) and aminopropyl SPE columns. A similar SPE cleanup has been reported for the determination of pesticides in fruits, vegetables, and milk (Sheridan and Meola, 1999) and low-moisture nonfatty products (Schenck and Howard-King, 1999).

Table 3. Incurred Pesticide Residues Recovered from Eggs after Dosing Hens on Nine Consecutive Days with Gelatin Capsules at an Equivalent Feed Concentration of 83 μg/g Chlorpyriphos-methyl

days after first dose	chlorpyriphos-methyl[a] (ng/g)	days after first dose	chlorpyriphos-methyl[a] (ng/g)
0	trace	5	52
1	12	6	59
3	44	7	54

[a] Single determination.

Table 4. Incurred Pesticide Residues Recovered from Eggs after Dosing Hens on Nine Consecutive Days with Gelatin Capsules at an Equivalent Feed Concentration of 63 μg/g Dimethoate

days after first dose	dimethoate[a] (ng/g)	omethoate[a] (ng/g)
0	40	20
1	30	20
2	40	40
3	30	50
4	50	90
6	40	110
7	60	60
8	20	70
9	30	60
10	7	11
12	1	1

[a] Single determination.

The SPE cleanup removed most of the coextracted pigments, resulting in a clear, colorless extract.

Although very few chromatographic interferences were observed when using the FPD for analysis of OPPs, further cleanup of the extract was required before trace levels of OCPs could be determined using the ECD. This cleanup was accomplished using a previously reported Florisil SPE column cleanup (Schenck et al., 1996) to remove the matrix interferences.

The presence of pesticide residues in feed is an important source of pesticide contamination in eggs. Waldron and Naber (1974) showed that the rate of accumulation of pesticides in eggs is related to the pesticide concentration in the feed. The pesticide residues most commonly found in feed samples analyzed in one of our laboratories have been malathion and chlorpyriphos-methyl, the maximum levels found being ~15 and ~0.03 μg/g, respectively (unpublished data, F. Schenck). Four hens were dosed with chlorpyriphos-methyl, dimethoate, malathion, or methoxychlor. Neither malathion nor malathion oxygen analogue was found in the eggs (limits of detection < 0.5 ng/g) when the hens were dosed at a level that would be equivalent to the ingestion of a feed containing an unusually high level of malathion, 500 μg/g for 3 days. Similarly, dosing the hens with a feed equivalent of 17 μg/g methoxychlor for 2 days resulted in no detectable methoxychlor residues in the eggs. Waldron and Naber (1974) also reported no accumulation of methoxychlor in eggs during a feeding study in which hens were fed 1.0 μg/g methoxychlor for 32 weeks. Dosing the hens with chlorpyriphos-methyl resulted in chlorpyriphos-methyl residues being found in the eggs (see Table 3). Dosing the hens with dimethoate resulted in both dimethoate and omethoate residues being found in the eggs (see Table 4).

Both polar and nonpolar pesticides can be efficiently recovered from eggs using the SPE method described. An excellent cleanup is accomplished with a minimal number of steps, and detection limits down to the low parts per billion range can be easily achieved.

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Received for review February 1, 2000. Revised manuscript received July 23, 2000. Accepted July 28, 2000.

JF000142C