Simplified Extraction and Cleanup for Determining Organochlorine Pesticides in Small Biological Samples

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In 1968, the senior author developed a microanalytical procedure for measuring organochlorine pesticide residues in very small biological samples (1 g or less). We have routinely used this procedure since 1969 and find it suitable for analyzing all sizes and types of samples. Rather than the conventional steps of Soxhlet extraction, macro-partition, and Florisil liquid adsorption cleanup in a column, our method uses simple shake extraction, micro-partition, and Florisil adsorption cleanup in the tube.

Because the technique permits rapid analysis, one person can prepare and analyze 5 to 10 samples per day. Extraction efficiency and recoveries are good to excellent for organochlorine pesticides and similar compounds (including polychlorinated biphenyls) and for at least three organophosphate pesticides (malathion, parathion, and methyl parathion).

DESCRIPTION OF THE METHOD

We have analyzed plants and plant parts, animal tissues ranging from single organs to whole-body homogenates, blood and blood fractions, milk, urine, and eggs. All are handled alike except that the ratios of the sodium sulfate desiccant and the extraction solvent differ for solid and liquid samples. Solids such as tissue are prepared for extraction by thoroughly grinding large samples in a blender or small samples in a mortar with five parts sodium sulfate to one part sample (w/w). Liquids such as blood are placed in a mortar and mixed with eight parts sodium sulfate to one part sample (w/w); the mixture is chilled in the mortar until it becomes brittle (about 15 min at -30° C) and is then finely ground with a pestle.

Extraction

All or part of the ground sodium sulfate mixture, which represents 0.1 to 10.0 g of starting sample, is extracted by vigorous shaking for at least 10 min with 20% acetone in isooctane (v/v) at a ratio of 10 ml solvent per 1-g solid sample equivalent or 5 ml solvent per 1-ml liquid sample equivalent. After solids have settled, an aliquot of the extract is placed in a centrifuge tube and spun at about 1,800 rpm for 3 or 4 min. If results are to be reported on a lipid-weight basis, a small portion of this clarified extract is withdrawn and evaporated at 70° C in a tared beaker to

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determine lipid equivalent. An amount of the clarified extract, which represents 0.4 g of starting sample, is transferred to a 15- x 100-mm culture tube and reduced to dryness with a gentle stream of clean, dry air.

Liquid-Liquid Partition

To the tube containing the dry sample residue, 4.0 ml of isooctane-saturated acetonitrile and 2.0 ml of acetonitrile-saturated isooctane are added. After the tube has been shaken vigorously for about 5 min, it is centrifuged at about 1,800 rpm until phase separation is complete. The entire lower layer (acetonitrile) is withdrawn with a 5-ml long-needled syringe and placed in a 20- x 125-mm culture tube. The isooctane layer is partitioned as before with another 4.0 ml of isooctane-saturated acetonitrile, and this acetonitrile layer is combined with the first one. To the 8.0 ml of combined acetonitrile solution, isooctane is added at a volume determined by the room (solvent) temperature: 3.3 ml is added if the room is between 62° and 70° F; 3.2 ml, between 72° and 78° F; and 3.1 ml, between 80° and 82° F. The tube is then nearly filled with a solution of 0.5% sodium sulfate in water (w/v), capped, and shaken vigorously for 3 to 4 min. When the layers have separated, the upper (isooctane) layer should have a volume of 4.0 ml (the effect of temperature on the solubility of isooctane in acetonitrile has been accounted for in the previous step); 2.0 ml of this isooctane layer is withdrawn, placed in a 15- x 100-mm culture tube, and reduced just to dryness with a stream of air.

Florisil Adsorption

To this dry residue, 0.4 ml of 5% methanol in isooctane (v/v) is added, which yields a sample equivalent of 0.5 mg of sample per microliter. To this solution is added 0.2 g of Florisil (as received, already activated at 1,250° F). The Florisil is swirled in the solvent and then coalesced by immersing the bottom of the tube in an ultrasonic cleaner tank for a few seconds. After standing for a few minutes, the solvent is clear and ready for gas chromatography. For our routine analyses, we use electron-capture detection with two unlike columns (QF-1 and OV-1).

PROOFS OF THE METHOD

At various times, we have run formal tests to compare our method with Soxhlet extraction and to determine pesticide recoveries from spiked samples of tissue and blood serum.

Lipid Extraction Comparison

To demonstrate that lipid values obtained by our method are adequate, 26 samples of various tissues from different birds were ground with sodium sulfate and then divided. Part of each sample was extracted by Soxhlet [15% ethyl ether in petroleum ether (v/v)] for 6 hours, and the other part was extracted by our method. The

lipid content of individual samples ranged from 7.6 to 638 mg/g of tissue by our method and 6.3 to 643 mg/g by Soxhlet. The overall mean was 80.0 mg/g by our procedure and 78.4 by Soxhlet. Sign-test comparison showed that our method extracted more lipids than Soxhlet (significant at the 97.5% confidence level). However, such small differences would ordinarily have little practical significance, even when residues are reported on a lipid basis.

Pesticide Recoveries from Animal Tissue

Most of our analyses are for common organochlorine pesticides in animal tissues. We tested recoveries from our method for this kind of analysis by spiking whole-body chicken samples with DDE, DDT, endrin, and heptachlor epoxide. A whole dressed chicken (including heart, liver, and gizzard) was finely ground with a meat grinder, and five 10-g samples were taken and individually ground with sodium sulfate. Four of these samples were spiked with all four pesticides at one of four 10-fold increments from 0.2 to 200 ppm. The fifth sample was used for a blank control. After extraction, four aliquots of the extraction solution were taken from each sample, carried through the cleanup steps, and analyzed by our usual chromatographic methods. Recoveries (Table 1) averaged 90-100% for 0.2- and 2-ppm samples and 84-95% for 20- and 200-ppm samples.

Pesticide Recoveries from Serum

Most of the blood and serum samples we analyze are from laboratory experiments in which a few known pesticides have been used; consequently, these samples do not require cleanup by partition and Florisil adsorption. Recoveries were tested with spiked serum samples processed by this further abbreviated procedure. Three serum samples from untreated mallards were each divided into five parts, spiked with DDE at 0.001, 0.005, 0.01, 0.1, and 10 ppm, and ground with sodium sulfate. Each subsample was then extracted with acetone-isooctane and centrifuged, and an aliquot of the supernatant was chromatographed. Recoveries (Table 2) averaged 65% at 0.001 ppm but 89% or better at 0.005 ppm and above.

ANALYTICAL PRECISION

As part of our analytical quality control program, we routinely repeat every ninth sample as a check for error. A statistical procedure has now been developed (K. M. Stahl, in prep.) by which these duplicate analyses can be used to appraise our overall analytical precision. Briefly, the mean residue concentration ($\bar{C}$) and the individual standard deviation (S) are calculated for each duplicate pair. Within series (similar sample types, same chemical measured), the standard deviation (S values) usually increases with the mean concentration ($\bar{C}$ values). To determine if this increase is linear, we check the significance of the linear correlation of S to $\bar{C}$. If the correlation coefficient (r) is significant, we calculate the regression equation ($S = a + b\bar{C} + e_i$) for the series.

TABLE 1

Pesticide Recoveries from Spiked Whole Chicken
(Four Replicates per Level)

Pesticide	Level of spike (ppm)	Recovery		
		Mean (ppm)	Standard deviation (ppm)	Mean % of spike recovered (±95% confidence interval)
DDE	0.0 (blank)	0.02	0.004	
	0.2	0.18	0.016	90 ± 11
	2.0	1.80	0.020	90 ± 1
	20.0	17.40	1.80	87 ± 16
	200.0	168.0	16.40	84 ± 12
DDT	0.0 (blank)	0.0	0.0	
	0.2	0.20	0.016	100 ± 10
	2.0	1.88	0.060	94 ± 4
	20.0	19.0	2.28	95 ± 19
	200.0	186.0	21.60	93 ± 14
Endrin	0.0 (blank)	0.0	0.0	
	0.2	0.18	0.014	90 ± 10
	2.0	1.88	0.060	94 ± 4
	20.0	18.0	1.43	90 ± 12
	200.0	196.0	16.5	88 ± 11
Heptachlor epoxide	0.0 (blank)	0.0	0.0	
	0.2	0.18	0.010	90 ± 7
	2.0	1.88	0.028	94 ± 2
	20.0	17.8	1.18	89 ± 10
	200.0	172.0	18.4	86 ± 13

TABLE 2

DDE Recoveries from Spiked Mallard Serum (Three Samples per Level)

Level of spike (ppm)	Recovery		
	Mean (ppm)	Standard deviation (ppm)	Mean % of spike recovered (±95% confidence interval)
0.001	0.00065	0.00011	65.0 ± 40.0
0.005	0.00446	0.00011	89.3 ± 6.1
0.01	0.00949	0.00023	94.9 ± 5.9
0.1	0.0921	0.00189	92.1 ± 5.1
10.0	9.57	0.1443	95.7 ± 3.7

The regression coefficient (b), when multiplied by 100, gives the overall percent coefficient of variation (CV) for the series; using the following formula, we can then estimate how far, in percent, residue values ascertained for a sample can be expected to vary from the pair mean ($\overline{C}$):

$$P = CV\ (t_{0.95,N-2}) \ /\ \sqrt{n}$$

where
- P = percent variation of individual C values about $\overline{C}$ at the 95% confidence level
- CV = overall coefficient of variation
- t = 95% probability value, taken from Student's t table, for N-2 degrees of freedom
- N = total number of pairs in the series
- n = 2, since each is a pair determination

We use the resulting P value to measure the precision of our analytical method. For example, P was calculated for two recent series of duplicate DDE analyses--a set of 20 bird and egg samples and a set of 54 mallard serum samples. Both sets produced significant correlations (r) of S to $\overline{C}$. The 20 bird and egg samples, over a residue range of 0.1 to 100 ppm, showed an r of 0.9734, an overall CV of 7%, and a P value of 9.9%. The 54 serum samples, over a range of 0.001 to 3.2 ppm, showed an r of 0.805, an overall CV of 4%, and a P value of 5.3%. These results mean that, for any sample, 95% of all duplicated values should vary from the pair $\overline{C}$ value already determined by no more than ±9.9% for tissue and egg samples and no more than ±5.3% for serum samples. Thus, our analytical method is not only rapid and efficient but appears quite precise even in routine use.