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Solid-Phase Extraction Method for the Quantitative Analysis of Organochlorine Pesticides in Wildlife Urine

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Abstract

A gas chromatographic method for the analysis of nine organochlorine pesticides in wildlife urine is described. Reversed-phase solid-phase extraction is utilized to extract the organochlorine pesticides from urine. The pesticides are recovered by elution with hexane–ethyl ether (1:1) and quantified by gas chromatography with electron-capture detection. Method detection limits range from 1.4 to 2.7 µg/L. Mean recoveries for all pesticides are 90.6%.

Introduction

The Rocky Mountain Arsenal (RMA) National Wildlife Refuge constitutes 6,900 hectares of short grass prairie located approximately 16 km northwest of downtown Denver, CO. In addition to serving as a weapons production facility, portions of the site have been leased by private corporations and used for the production of organochlorine pesticides. Such activities began in the late 1940's and were terminated by 1983 (1). Waste disposal and storage practices typical for that time have contaminated areas of the refuge with a variety of chemicals. Numerous chemicals resulting from human activity have been identified on this Superfund National Priorities listed site (2).

Based on a number of factors including potential toxicological effects, chemical properties, environmental stability, and frequency of detection on the site, the top five chemicals of concern identified by both the U.S. Army and the U.S. Environmental Protection Agency are the organochlorine pesticides dieldrin, aldrin, endrin, 1,1'-(2,2,2-trichloroethylidene) bis [4-chlorobenzene] (DDT), and 1,1'-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (DDE). Due to the high frequency of detection in plants and animals sampled from the site, dieldrin has been determined as the primary chemical of concern. To evaluate the effects of chemical contamination of the RMA Wildlife Refuge on wildlife, the U.S. Fish and Wildlife Service (USFWS) is conducting wildlife biomonitoring studies primarily for dieldrin. It is hoped that these studies will contribute to remediation strategies. Optimally focusing resources on cleanup activities will assure the safety of wildlife while preventing the unnecessary cleanup of areas that do not pose a significant threat.

Potential absorption of dieldrin by wildlife is typically oral or dermal exposure and to a lesser extent, via inhalation. Oral median lethal doses (LD_{50}s) for mouse, rat, and guinea pig range from 38 to 49 mg/kg whereas dermal LD_{50}s range from 40 to 120 mg/kg (3). The oral and dermal LD_{50}s are quite similar, so absorption of dieldrin is likely to be similar for both routes of exposure. Following an oral dose of 693 µg ^{14}C-dieldrin, ^{14}C urinary excretion over 8 days accounted for approximately 6% of the initial dose. Depending on the day of sampling, dieldrin accounted for 7–41% of the dieldrin-derived urinary residues. Dieldrin and dieldrin metabolites were also detected in adipose, kidney, and liver (4). Additional experiments with mice and rats indicated urinary excretion of dieldrin and dieldrin metabolites and that the nature of metabolites was species- and sex-specific (5–7).

In the development of a biomonitoring procedure for dieldrin exposure that is adaptable to multiple wildlife species, the anal-

Table I. Method Validation Mean Percent Recovery Data for Fortified Canine Urine

	Fortification Level (µg/L)						
Compound	25	50	100	250	500	Overall mean	Standard deviation
Lindane	60	85	90	97	97	85.8	15.2
Aldrin	49	48	55	72	84	61.6	15.7
Heptachlor epoxide	67	86	114	120	119	101.2	23.6
trans-Chlordane	70	76	89	110	112	91.4	19.2
cis-Chlordane	76	79	92	113	114	94.8	18.1
p,p' - DDE	68	66	104	96	95	85.8	17.5
Dieldrin	81	82	89	115	115	96.4	17.3
Endrin	75	81	122	119	118	103	22.9
p,p' - DDT	72	75	106	112	112	95.4	20.2

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ysis of urine for dieldrin is promising. Furthermore, collection of urine from captured wildlife is nonintrusive. To develop a model for relating urinary dieldrin levels with toxicity, captured wildlife will initially be dosed with dieldrin, and urinary excretion of dieldrin will be determined over time. Relationships between gross pathology, histopathology, various biochemical and physiological endpoints, residue levels, urinary dieldrin levels, and dose levels will need to be determined. The development of an analytical method to quantitate dieldrin and other organochlorine pesticides in wildlife urine is critical to the development of the model and implementation of this biomonitoring program. This paper reports the successful development and implementation of such an analytical method.

Experimental

Materials

Neat organochlorine pesticide standards were obtained from Chem Service (West Chester, PA). Ether (anhydrous +99%) was obtained from Aldrich Chemical (Milwaukee, WI). Pesticide residue-grade acetone and hexane was from Fisher Chemical (Fair Lawn, NJ). One gram, 6 mL, IST C_{18} (endcapped) solid-phase extraction (SPE) columns and Vacmaster sample processing stations were from Jones Chromatography (Lakewood, CO). Gas chromatography (GC) expendables including inlet liners, silanized glass wool, and gold inlet seals were from Restek (Bellefonte, PA).

Standard preparation

Stock standards were prepared from neat materials, dissolved in acetone (1000 µg/mL), and diluted in acetone to prepare standard solutions for fortification (10 µg/mL). Instrument calibration stock (1000 µg/mL) and diluted (10 µg/mL) standards were prepared in hexane.

Sample fortification

Controlled canine urine collected from a single domestic dog was fortified with a mixed standard containing lindane, aldrin, heptachlor epoxide, trans-chlordane, cis-chlordane, p,p'-DDE, dieldrin, endrin, and p,p'-DDT. For method validation, control urine was fortified at 5 levels: 25, 50, 100, 250, and 500 µg of each compound per liter. For daily positive quality control samples, controlled urine was fortified with each compound at 250 µg/L. Daily blank quality control samples and actual wildlife urine samples were fortified with lindane only (surrogate standard) at 250 µg/L.

Table II. Method Detection Limits and PMRMA* Method Reporting Limits in Canine Urine

Compound	Detection Limit (µg/L)	Reporting limit (µg/L)
Lindane	2.7	50
Aldrin	2.7	111
Heptachlor epoxide	1.6	29
trans-Chlordane	1.4	55
cis-Chlordane	1.3	43
p,p'-DDE	1.7	92
Dieldrin	1.5	114
Endrin	1.6	121
p,p'-DDT	1.5	204

* Program Manager, Rocky Mountain Arsenal.

Sample preparation

SPE columns (C_{18}) were placed on the sample processing station and preconditioned with 3 × 6-mL aliquots of deionized water; the last aliquot eluted to the top of the column packing only. Frozen urine samples were brought to room temperature, and a 3-mL aliquot was centrifuged for approximately 5 min at 4000 g. A 1.0-mL aliquot of the centrifuged urine was transferred to the SPE column using a Hamilton (Reno, NV) 1000-µL syringe. The sample was then spiked with the appropriate standard solution. After 1 h of equilibration at room temperature, the stopcock was opened, and the urine eluted through the column at ambient pressure. When the elution had stopped, the remaining urine was eluted through the column with a gentle vacuum (–2 in. Hg from actual manifold gauge). The eluate was discarded, and the column was dried for 20 min under a full vacuum (–20 in. Hg). After drying, the manifold needles were rinsed with hexane to remove any dried urine, and the collection tubes were removed and replaced with 10-mL tubes that had been calibrated to a 1.0-mL volume with hexane. The organochlorine pesticides were eluted with 3 × 3-mL aliquots of 1:1 hexane–ethyl ether, and the final amount of solvent in the column was removed under a gentle vacuum. The extracts were concentrated to less than 1.0 mL under a gentle stream of nitrogen in a fume hood, equilibrated to room temperature, and brought to a final volume of 1.0 mL in hexane. The samples were then capped, vortexed, and transferred to GC vials for pesticide quantitation via GC analysis.

GC

The Hewlett-Packard (HP) GC system consisted of a 5890 GC (250°C inlet) equipped with electronic pressure control, dual electron-capture detectors (350°C), and dual 7673A auto samplers. The system was PC-controlled utilizing HP ChemStation software. The carrier gas was helium (25 cm/s), and the makeup gas was argon–methane (60 mL/min). The quantitation column was 30 m × 0.25-mm i.d. fused-silica, HP-5 crosslinked 5% phenyl methyl–silicone stationary phase, 0.25-µm film thickness (Hewlett-Packard). The confirmation column was 30 m × 0.25-mm i.d. fused-silica DB-17 bonded stationary phase with 0.15-µm film thickness (J&W Scientific).

The oven temperature program for quantitation and confirmation was as follows: 50°C for 0.25 min, 60°C/min to 100°C, 30°C/min to 190°C, held for 2 min, 10°C/min to 300°C, and held for 5 min. The electronic pressure program for the quantitation column was 80 psi for 2 min, 99 psi/min to 16 psi, and held for 23 min. The confirmation column inlet pressure was held at a constant 16 psi throughout the run. A 1.0-µL injection volume was used for the quantitation column with a single taper 4-mm-i.d. inlet liner packed with deactivated glass wool.

A 2-µL injection volume was used on the confirmation column, which utilized a double taper 4-mm-i.d. injection liner.

Method validation

The linearity of the detector response was determined by linear regression analyses of 5-point calibration curves ranging from 25 to 500 µg/L (response versus mass of analyte) for each analyte. After achieving regression coefficients greater than or equal to 0.99, linear regression equations were calculated and used to quantitate analytes in samples. Fortified control canine urine (5 levels) was cleaned up via the SPE procedure and analyzed by GC, and recovery percentages were determined for each analyte at each fortification level on two consecutive days. Method limits of detection (MLODs) were single-point calculated from the chromatogram of the 25-µg/L fortified urine. MLODs were calculated as the quantity of analyte required to give a response of 3 times the baseline noise at the expected retention time of the analyte in the chromatogram of nonfortified urine. Method reporting limits (MRLs) were calculated by the technique of Hubaux and Vos (8) and the requirements of the Program Manager RMA (9).

Quality control

All wildlife urine samples were fortified with 250 µg lindane/L during sample preparation. Positive control (fortified with all analytes at 250 µg/L) and blank control urine samples (fortified with lindane at 250 µg/L) were also prepared and analyzed with each group of 20 wildlife urine samples.

To assure constant instrument performance prior to the GC analysis of any sample, endrin and DDT degradation was shown to be less than or equal to 20% for each compound and less than or equal to 30% for both compounds as indicated by the analysis of a 250-µg/L endrin and DDT standard. Also, a 250-µg/L instrument calibration check standard was analyzed at the beginning, after every 10 samples, and at the end of each analytical run. The response for the majority (two thirds) of the analytes and all compounds positively identified in the samples was required to match the response of the calibration curve within 25% throughout the analysis.

Method performance was monitored and documented by tracking the recoveries of five of the fortified analytes: aldrin, dieldrin, endrin, p,p'-DDT and *trans*-chlordane. Surrogate recoveries were used to monitor individual sample extraction proficiency and instrument performance throughout the entire analysis sequence. In addition to analysis on the quantitation column, extracts of all urine samples found to contain organochlorine pesticides were confirmed by GC analysis on the confirmation column.

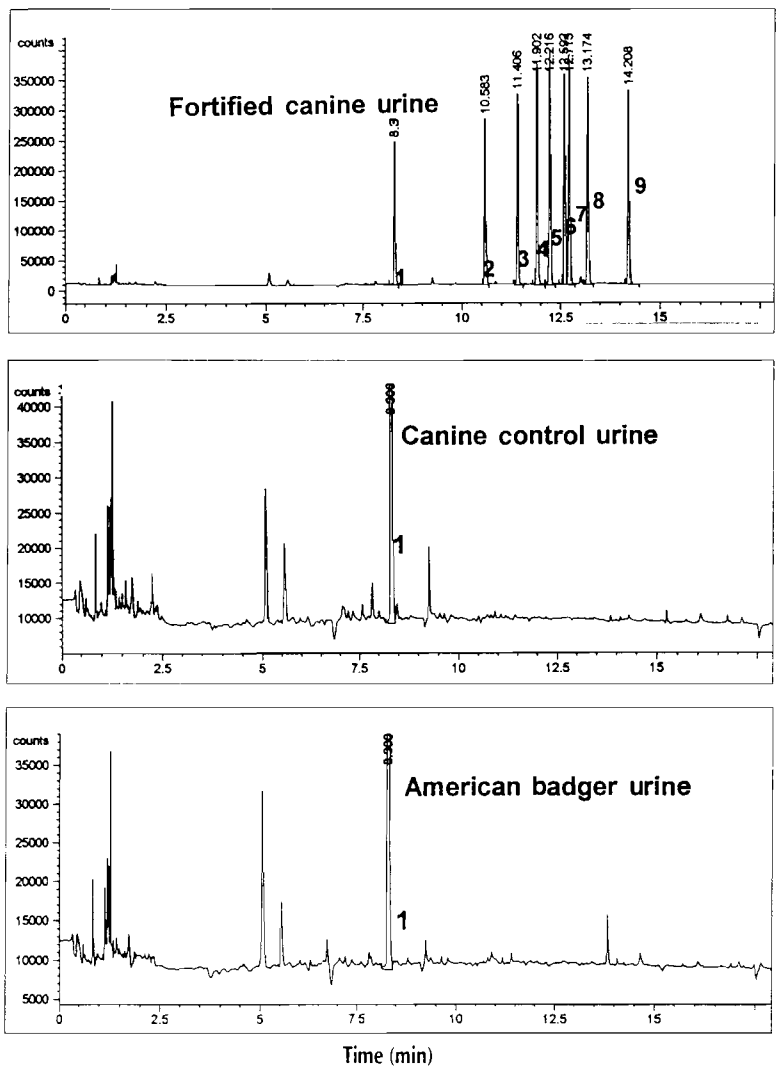

Figure 1. Chromatograms of 250 ng/mL fortified canine urine, canine control urine, and American badger urine. Peaks: 1, lindane (surrogate); 2, aldrin; 3, heptachlor epoxide; 4, *trans*-chlordane; 5, *cis*-chlordane; 6, p,p'-DDE; 7, dieldrin; 8, endrin; and 9, p,p'-DDT.

Results and Discussion

Due to the commercial unavailability of wildlife urine matrices, canine urine was utilized. Control canine urine proved to be an acceptable matrix for method development and validation, as indicated by the chromatograms presented in Figure 1. The chromatograms from the analyses of canine urine and American badger urine (*Taxidea taxus*, trapped at an uncontaminated site) were quite similar. Both chromatograms were free of interfering peaks at the retention times of the organochlorine pesticides of interest, as indicated by the chromatogram of fortified canine urine.

The results of the method validation experiments are presented in Table I. For dieldrin, the analyte of primary concern, the mean recovery was 96.4%, and the standard deviation was 17.3%. Mean recoveries of the other analytes of primary concern, aldrin, endrin, DDT, and DDE, were 61.6% ± 15.7, 103% ± 22.9, 95.4% ± 20.2, and 85.8% ± 17.5, respectively. The mean recovery of lindane, the compound added to all samples as a surrogate standard, was 85.8% ± 15.2. This is sim-

Table III. Recovery Data of Surrogate and Fortified Analytes From Wildlife and Canine Urine

Compound	250 µg/L Fortification Replicate									Mean	Standard Deviation
Lindane*	76	69	88	76	79	90	70	75	81	78	7.2
Aldrin	33	39	47	65	62	50	49	44	92	53	17.6
Heptachlor epoxide	88	85	97	94	111	102	91	97	117	98	10.5
t-Chlordane	80	79	86	90	99	89	85	85	99	88	7.2
c-Chlordane	79	81	86	93	100	91	88	85	98	89	7.2
p,p'-DDE	65	70	68	77	86	74	75	68	82	74	7.0
Dieldrin	83	85	90	95	103	93	90	87	101	92	6.8
Endrin	94	94	95	97	109	99	96	94	109	99	6.1
p,p'-DDT	69	na	83	94	100	88	88	83	90	87	9.1

* Each lindane replicate is the average of 20 fortified badger urine samples.

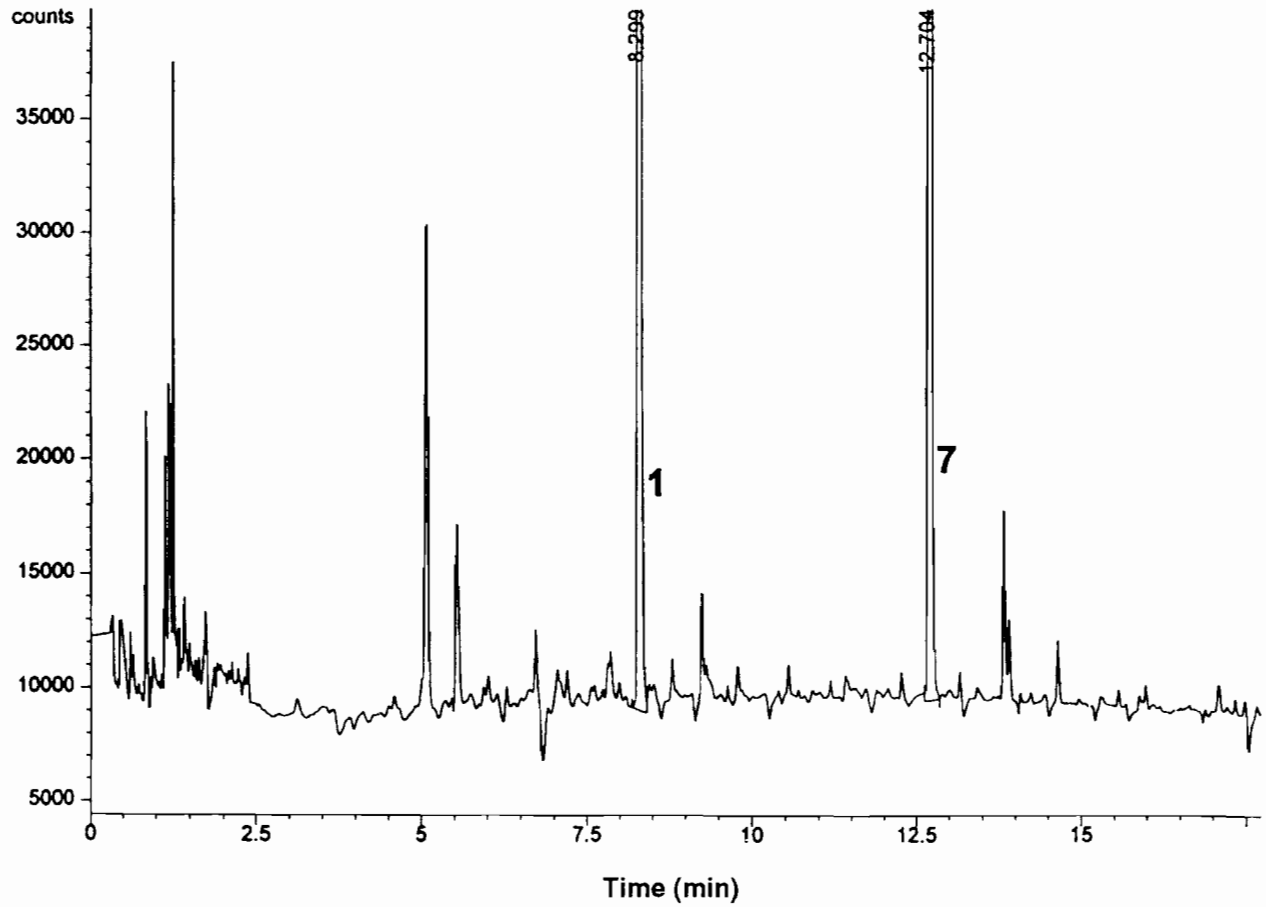

Figure 2. Chromatogram of American badger urine sample containing lindane surrogate (peak 1) and an incurred dieldrin residue (peak 7).

ilar to the 90.6% ± 12.4 mean recovery for all compounds, indicating the suitability of lindane as a surrogate standard for these analyses. MLODs are presented in Table II. The MLOD for dieldrin was 1.5 µg/L. The MLODs for the other analytes of primary concern ranged from 1.3 µg/L for *cis*-chlordane to 2.7 µg/L for aldrin. Similar levels of detection were achieved on quantitation and confirmation columns.

As indicated by the recovery data presented in Table III, this method proved sufficiently rugged for the analyses of urine samples collected from animals on the RMA National Wildlife Refuge. During the analyses of 180 samples, recoveries of the lindane surrogate were quite consistent, as indicated by the average recovery and standard deviation of 78% ± 7.2. Recoveries of dieldrin from the fortified control urine analyzed with each lot of 20 samples was 91.9% ± 6.8. Dieldrin was detected in urine collected from badgers that had been trapped in uncontaminated areas and dosed with dieldrin and from badgers trapped within contaminated areas of the RMA National Wildlife Refuge. This method was utilized for the creation of an accurate model to describe dieldrin dose versus urinary excretion and proved useful in biomarker studies of badgers with known chronic exposure (10). The method showed limited applicability to large field studies based on the uncertainty of exposure times of collected animals and difficulties in physical sample collection (10).

A chromatogram from the analyses of urine collected from a badger trapped on the Rocky Mountain Arsenal is shown in Figure 2. The presence of dieldrin is indicated by the peak at 12.704 min and was confirmed by simultaneous analysis on the confirmation column.

Using a 40- and 20-port sample processing station, an analyst typically prepared 40 samples and four quality control samples for GC analyses in 4 h. These 40 samples were usually analyzed by GC in two lots of 20; each lot was analyzed on separate days. Each sample required 9 mL of ether and 10 mL of hexane; less than 1 mL of hexane remained for disposal after analysis.

Conclusion

This reversed-phase SPE–GC method with electron-capture detection for the analysis of organochlorine residues in wildlife urine proved to be sufficiently rugged for the analysis of nearly 200 wildlife urine samples. The quality control measures presented permitted continuous monitoring of method performance and assured the generation of scientifically valid data that can be used to influence the prioritization of resources for the cleanup of a Superfund site.

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References

1. Biota Remedial Investigation. Final Report. Volume I. Environmental Science and Engineering, Denver, CO. Prepared for the Program Manager, Rocky Mountain Arsenal, 1989.
2. R.L. Stollar and Associates. Comprehensive monitoring program: Biota Annual Report, 1990 and Summary Report, 1988–1990. Prepared for the Program Manager, Rocky Mountain Arsenal, 1992.
3. WHO. World Health Organization Report on Aldrin / Dieldrin. Environmental Health Criteria 1991, 1989.
4. D.H. Hutson. Comparative metabolism of dieldrin in the rat (CFE) and in two strains of mouse (CF1 and LACG). *Food and Cosmetic Toxicol.* **14:** 577–91 (1976).
5. M.K Baldwin, J. Robinson, and D.V. Parke. A comparison of the metabolism of HEOD (dieldrin) in the CF1 mouse compared with the CFE rat. *Food and Cosmetic Toxicol.* **10:** 333–51 (1972).
6. H.B. Mathews, J.D. McKinney, and G.W. Lucier. Dieldrin Metabolism, Excretion and Storage in Male and Female Rats. *J. Agr. Food Chem.* **19:** 1244–48 (1971).
7. D.F. Heath and M. Vandekar. Toxicity and Metabolism of Dieldrin in Rats. *Brit. J. Industr. Med.* **21:** 269–79 (1964).
8. A. Hubaux and G. Vos. Decision and Detection Limits for Linear Calibration Curves. *Anal. Chem.* **42**(8): 849–55 (1970).
9. Rocky Mountain Arsenal, Chemical Quality Assurance Plan, Version 1, September 1993.
10. D.J. Hoff. Integrated Laboratory and Field Investigations Assessing Risk to American Badgers on the Rocky Mountain Arsenal National Wildlife Refuge. Ph.D. Dissertation, Clemson University, Clemson, SC, 1997.

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