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Determination of Dieldrin Concentrations in Recycled Cattle Feed and Manure by Liquid Chromatography

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Recycling of animal manure as feed is an agricultural resource management practice which impacts directly on animal health. The recovery of protein and cellulosic feed materials from cattle manure has been intensively studied. A variety of commercial methodologies have been patented, and reviewed (Gilles 1978). One such process developed by Ceres Ecology Corporation (US patent 4018899) produces three products from manure: a water insoluble cellulosic fiber material consisting mainly of undigested plant material, a water soluble high protein material, and an inedible material suitable for use as a soil amendment. The fiber and the protein, called C-I and C-II, respectively, have been evaluated as feed ration ingredients for cattle, sheep, pigs, poultry, and other animals (Harper and Seckler 1975). An important consideration in their use is the potential presence of pesticide residues. Organochlorine insecticides, including dieldrin, are globally distributed pollutants, and thus dieldrin residues can appear in feeds produced by recycling manure.

In this work we report the analysis for dieldrin by high performance liquid chromatography (HPLC)in feed, manure, and C-I and C-II products. Reversed phase HPLC has been used for the determination of other organochlorine pesticides (Sieber 1974; Kvalvag et al. 1979; Wolkoff and Creed 1981). Although gas chromatography (GC) is more sensitive for dieldrin (Cook and Ober 1980), HPLC is used because sample preparation is simple and rapid, the analysis is specific and has adequate minimum detectability for the concentrations involved (sub-ppm), and HPLC is a viable alternate method which can be automated for the routine analysis of large numbers of samples.

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MATERIALS AND METHODS

Samples were obtained during an independent 28 week feeding study (Smith 1981) in which a herd of 36 selected Holstein heifers was split into three equal groups. The control group was fed a diet of 30% oat hay, 25% alfalfa, 41% barley, 3.7% cottonseed meal, and 0.3% trace mineral salt. Test group A received a similar diet, except that C-I was substituted for the oat hay. Test group B received a diet which contained no oat hay, 5% alfalfa, 45% C-I, 8.5% cottonseed meal, and 0.2% limestone; the barley and trace mineral percentages were the same. Fresh fecal samples were collected from each group on an approximately weekly basis and refrigerated until analyzed. Feed samples were collected whenever a fresh batch was formulated, and at other random times. These were stored in new polyethylene bags for analysis. Samples of process manure and the C-I and C-II products were taken at the production facility on days these materials were produced.

Samples were dried at 40°C and 40 cm water vacuum for 10 h. The dried samples were homogenized in a small laboratory blender, and each sample (17 g) was extracted twice with 50 mL of chloroform. The chloroform extracts were combined, evaporated to exactly 10 mL, and stored in brown,glass,screwcap bottles. Small (one mL) aliquots were removed just prior to analysis and filtered through 0.45 um membrane filters (Gelman Scientific, Ann Arbor MI).

An isocratic liquid chromatograph with a UV detector set at 234 nm (Waters Associates , Milford MA) was fitted with an Ultrasil ODS column (4.6mm x 25 cm,10 um particle size; Alltech Associates, Deerfield IL). Elutions were made by using methanol + water (9:1, v/v) at 22°C and a flow rate of 2.1 mL/min. A 75-uL sampling loop was used to inject the chloroform extracts or dieldrin standards . Dieldrin was identified by its retention time, and quantitated by measuring peak heights.(Dieldrin identity in selected samples was confirmed by GC/MS). Duplicate injections were used. Calibration curves were prepared by using solutions containing from 0.15 to 1.5 ug/uL dieldrin, which was the range observed in the sample extracts. Calibrations were corroborated by standard addition techniques by using feed sample extracts to rule out coelution of extraneous interfering compounds.

The recovery of dieldrin from feed and feces was determined using stored or freshly thawed samples fortified with sufficient dieldrin in dichloromethane solution to give 10 and 50 ppb on a dry weight basis.

After drying under vacuum, the fortified samples were extracted. Independently, the recovery was also checked with unfortified samples by using exhaustive Soxhlet extraction (Mumma et al. 1966) for 12 h with 1:1 (v/v) chloroform/methanol for verification.

RESULTS AND DISCUSSION

The simple chloroform extraction procedure recovers better than 96% of the dieldrin added before drying, based on the results of studies with fortified feed and feces samples. These recoveries compare favorably with those of Santa Maria et al.(1986), Bottomley and Baker (1984), and Chang-Yen and Sampath (1984). The exhaustive Soxhlet extraction procedure, which is much more lengthy, gave only slightly better (3-4%) recoveries. No significant differences were observed in the recoveries from feed or feces.

Dieldrin concentrations in duplicate unfortified samples were also treated by the two different extraction techniques to compare extraction efficiencies in the case where the dieldrin might be intimately bound within the matrix. One feed and one fecal sample from each experimental group was dried, blended, and divided into equal portions for extraction. Each extract was analyzed in triplicate by HPLC. The exhaustive extraction gave results which were 3 to 4% larger, on the average, but with no increase in precision between replicates.

The calibrations were linear in the range studied. Calibrations were checked daily; the slope of the calibration curve showed a 2.9% coefficient of variation (CV) over a typical twenty day operating period. The reproducibility in peak height was evaluated by injecting a control feed sample containing 51 ppb dieldrin. The CV for peak heights for ten injections was 2.3%, and the CV for retention times was 0.81%. The lower limit of reportable residues by this method was 0.13 ug. This is an improvement over the reported limit of 1-15 ug of organochlorine pesticides for HPLC at 254 nm (Ogan et al. 1981). Our lower limit at 96% recovery corresponds to 7.9 ppb in a 17 g sample. Bottomley and Baker (1984) reported a 10 ppb detection limit for dieldrin in grain using GC with electron-capture detection. The use of a shorter UV detector wavelength and larger samples in HPLC thus provides comparable minimum detectability to the GC methods.

Figure 1 shows the typical peak shapes and separations for feed and feces samples when using the described conditions. The dieldrin concentrations in the injected

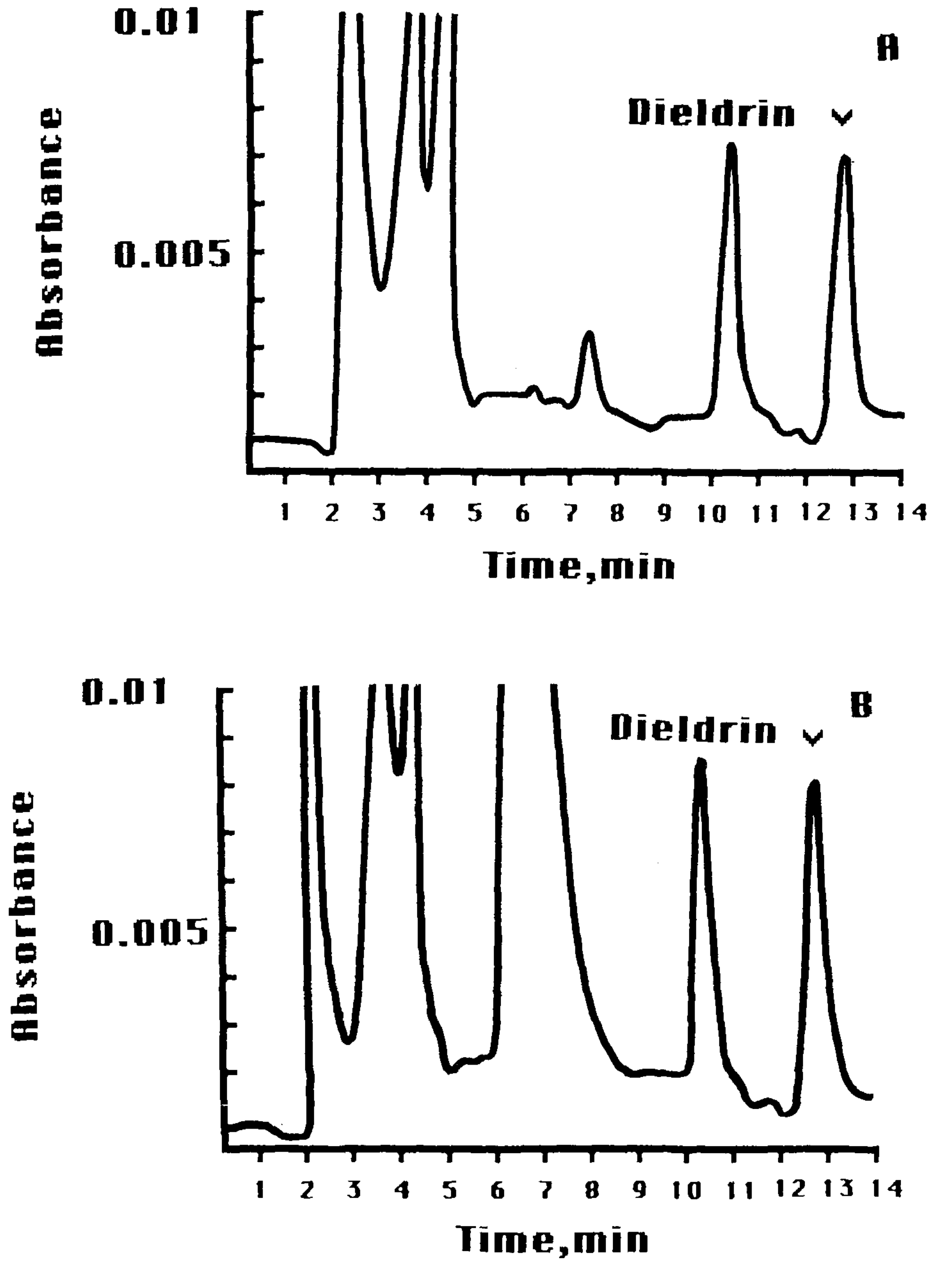

Figure 1.HPLC separation of dieldrin in samples from test group A. (A) Feed sample, 1.13 ug/uL; (B) feces sample, 1.15 ug/uL

aliquots are 1.15 and 1.13 ug/uL, respectively for the feed and feces extracts shown. Essentially baseline resolution of the dieldrin peak is observed. Thus a simple solvent extraction procedure followed by HPLC is suitable for the rapid quantitative analysis of dieldrin in feed and feces. Dieldrin concentrations in manure, C-I, and C-II were determined on four or more samples taken at the recycling plant. The values are as follows (ppb dieldrin ± % CV ,dry weight basis): manure , 32 ± 2.5%; C-I, 10 ± 5.7%; C-II, 20 ± 4.6%. Aldrin residues were not detected, and may not have been present, or possibly were converted *in vivo* to dieldrin (Kutz et al. 1979). Based on the above values, the C-I and C-II materials contain 31 and 63% respectively , of the dieldrin content of the manure from which they were extracted. Thus the recycling process partially removed dieldrin from these potential feedstuffs. They are suitable as feed ration ingredients, based on the FAO (1982) recommended acceptible dieldrin concentration limits of 20 ppb.They should not significantly impact the health of animals fed them in formulation, or the health of humans consuming products from those animals, based on published estimates (Eden 1951 ; El Ahraf et al. 1983).

The dieldrin concentrations found in feed and feces samples are shown in Table 1. The mean feed concentrations are comparable to the FAO/WHO 1982 maximum residue limits in grain of 20 ppb. However, grain (barley) was only 41% of the rations fed, and samples of the individual ingredients were not available for analysis. Feed samples were not taken on a weekly basis, but whenever a new batch was formulated, as indicated. Sample collection and storage problems caused the loss of several fecal samples. Mean values for each sample set and the mean fecal/feed dieldrin concentration ratio by group are also given. All of the feed rations for the test groups were formulated by using the same batch of C-I, prepared from dairy cattle manure prior to the start of the trials. No manure from the animals in this study was used. Dieldrin concentrations in manure are significantly higher than those in C-II, which in turn shows higher concentrations than does C-I. These differences are significant (p=.001). Fecal dieldrin concentrations did not change markedly during the feed trial. Groups A and B showed an early increase, but values never reached the concentrations found in the control group. The average concentration in control feces is significantly higher (p=0.05) for the control group than for either test group. Reasons for this are unclear. All animals were on the same water supply and penned in the same location. Their diets were similar, and differed mainly in the C-I, oat hay, and cottonseed proportions. However, dietary dieldrin concentrations were almost identical in the three feeds. A comparison

Table 1:Dieldrin Concentrations in Feed and Feces[a,b,c]

Control Group		Group A, 30% C-I		Group B, 45% C-I	
Feed	Feces	Feed	Feces	Feed	Feces
20 ± 1.1 (1)	71 ± 3.1 (1)	27 ± 1.3 (2)	18 ± 1.1 (1)	20 ± 1.2 (1)	11 ± 0.9 (1)
51 ± 1.3 (4)	72 ± 2.8 (2)	21 ± 1.2 (9)	12 ± 0.9 (2)	29 ± 1.4 (4)	72 ± 2.7 (2)
22 ± 1.1 (9)	63 ± 2.2 (3)	23 ± 1.3 (14)	67 ± 2.1 (4)	24 ± 1.3 (8)	40 ± 1.6 (4)
14 ± 0.9 (13)	73 ± 1.8 (5)	18 ± 1.0 (17)	62 ± 2.0 (5)	17 ± 1.1 (10)	35 ± 1.5 (5)
10 ± 0.9 (15)	76 ± 3.2 (8)		41 ± 1.7 (6)	44 ± 1.9 (13)	62 ± 2.0 (9)
10 ± 1.0 (17)	77 ± 1.7 (13)		55 ± 2.0 (8)	15 ± 1.1 (17)	48 ± 1.8 (14)
	51 ± 2.1 (16)		43 ± 1.9 (11)		37 ± 1.4 (18)
	35 ± 1.8 (18)		37 ± 1.4 (16)		
			46 ± 1.7 (18)		
Mean					
21 ± 1.0	64 ± 2.3	22 ± 1.2	47 ± 1.6	25 ± 1.3	44 ± 1.7
Fecal/Feed Ratio					
3.1 ± 0.5		2.1 ± 0.2		1.8 ± 0.2	

[a] parts per billion, dry weight basis, with standard deviation for three or more analyses

[b] trial week of sample collection shown in parentheses

[c] feed samples collected at formulation of new batches (2 to 6 week intervals)

of the ratio of average dieldrin concentration in feed to that in feces shows that the control animal ratio is highest, which suggests that the control group is able to eliminate dieldrin more efficiently than the test groups, given that the intake is similar. This deserves further study to determine if the C-I material in the test rations is responsible. Feeding studies (Smith 1981) showed weight gains for the B test group which were 15% less than controls, and the feed intakes for both groups A and B were 7% greater than controls. This suggests that the lower dieldrin concentrations per gram of feces in the test animals may be partly due to their consumption of larger quantities of the lower energy feed ration. This simple dilution effect can account for only about one third of the observed difference, at most.

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