Evaluation of Extraction and Cleanup Methods for Analysis of DDT and DDE in Green Alfalfa[1]

by GEORGE W. WARE and MELITA KOLB DEE
Department of Entomology
The University of Arizona, Tucson, Arizona

DDT and its metabolites are commonly found as trace contaminants in forage and hay grown in the irrigated mixed agricultural areas of Arizona. It has thus become the practice for many alfalfa growers and dairymen in these areas to have green alfalfa samples analyzed by commercial laboratories as prospective hay or for feeding as greenchop.

Whiting et al. (5) have compared the efficiency of various extraction methods and solvent systems for analysis of DDT and related materials in hay by electron capture gas chromatography. The most rapid and exhaustive hay extraction involved blending powdered hay for 10 minutes with chloroform:methanol (1:1).

Mumma et al. (2) indicated that the older extraction methods removed in the order of 60 to 70% of extractives from dry hay and improved most methods by an additional 12 hour Soxhlet extraction of green or dry material with methanol:chloroform (1:1).

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Wheeler et al. (4) found that with green plant material, the 12 hour Soxhleting was the most thorough extraction method as confirmed by using radio-labeled dieldrin.

It was the purpose of these experiments to compare several commonly used extraction and cleanup recoveries of green alfalfa for DDT and related materials to be analyzed by electron capture gas chromatography.

Experimental Procedure

The green alfalfa used in all portions of this study was obtained from a single field in Maricopa County. The field had not had pesticides applied to it during the two previous years, thus the residues reported represent inherent contamination. Approximately eight pounds of alfalfa were finely minced on a commercial salad chopper, mixed, samples withdrawn as the extraction method demanded, and held frozen until used.

Extraction Methods:

1. The 50 gm samples were minced in an omnimixer for 10 minutes with 300 ml of hexane:ethanol (2:1), and allowed to stand overnight (16 hours) in the quart blending jar at room temperature. The extract was then filtered through Whatman #30 paper into a 2 liter separatory funnel, washed three times with distilled water, and passed through a glass wool-sodium sulfate plug into a storage bottle. The remaining extracted

plant material was transferred to a Soxhlet apparatus with 300 ml of methanol:chloroform (1:1) and extracted for 16 hours. This second extract was then evaporated just to dryness in a steam bath and the extract redissolved in 100 ml of hexane. Five ml each of the two extracts were columned separately on a 4-inch Florisil column, and eluted with 200 ml of 15% diethyl ether in pentane.

1A. Extracts from the method described in 1 above, except that the column was eluted with 200 ml of 20% methylene chloride in petroleum ether.

2. The 50 gm samples were Soxhleted only, as in 1 above, with no previous extraction, and eluted from the column with 15% ethyl ether in pentane.

3. The 100 gm samples were minced for 10 minutes in an omnimixer with 200 ml of acetonitrile, filtered into a 2 liter separatory funnel and partitioned into 100 ml of petroleum ether, washing with 600 ml of water three times. The petroleum ether extract was passed through a glass wool-sodium sulfate plug into a storage bottle. The remaining plant material was Soxhleted as described in 1 above. Five ml aliquots of each were cleaned on Florisil using the 15% diethyl ether in pentane eluant, and analyzed separately.

4. The 50 gm samples were minced with 200 ml of 17.5% water in acetonitrile for 10 minutes, as suggested by Bertuzzi (1), filtered into a 2 liter separatory funnel and partitioned into 100 ml of petroleum ether, washing with 600 ml of water three times. The extract was dried and bottled as in 3 above. The remaining plant material was Soxhleted as described in 1 above. Five ml aliquots of each were columned, eluting with 15% diethyl ether in pentane, and analyzed separately.

4A. Extracts from method 4 above, except column eluted with 200 ml of methylene chloride in petroleum ether.

5. The samples were extracted and cleaned as in 4 above, but allowed to stand at room temperature 16 hours, before filtering and Soxhleting.

6. This procedure is identical to 1 above, except that the extract was filtered immediately following mincing and not allowed to stand overnight. Five ml aliquots of each were columned, eluted with 15% diethyl ether in pentane, combined and analyzed as one.

6A. Extracts from method described in 1 above, except that the column was eluted with 200 ml of 20% methylene chloride in petroleum ether.

7. The samples were extracted and cleaned as in 5 above, but hexane used in partitioning rather than petroleum ether.

8. The 15 gm samples were dried at room conditions for three hours, refluxed with 150 ml of acetonitrile (3), cooled and filtered. Of this, 20 ml were evaporated to dryness in a 50 ml beaker, and the residue dissolved in 5 ml of hexane. The Florisil column was eluted with 150 ml of 10% diethyl ether in petroleum ether which was discarded, then by 200 ml of 30% diethyl ether in petroleum ether.

All sample extracts were analyzed by electron capture gas chromatography.

Results and Discussion

In Table 1 are presented the mean results from the extraction and cleanup procedures, and their standard deviations. Method 1A resulted in much higher residue extraction than all others. This method involved hexane:ethanol extraction left standing 16 hours, followed by methanol:chloroform Soxhleting. In decreasing order of effectiveness are 1, 6A, 6, and 5. The remainder gave unsatisfactory recoveries.

The method of eluting the column with 20% methylene chloride in petroleum ether also enhanced recovery as seen in 1A, 4A and 6A, by an average of 18% beyond 15% diethyl ether in pentane.

The method resulting in the next highest recovery was 6A and 6, which utilized the same solvents but no time lapse between mincing and filtering.

TABLE 1

Residues (PPB) of DDT and related materials detected in green alfalfa by ECGLC from various methods of extraction and cleanup.

Extraction Method #	Replicates	DDE	o,p DDT	p,p' DDT	Total
1	4	92	162	706	960 ± 87
1A	4	96	192	888	1176 ± 141
2	5	34	70	272	375 ± 41
3	4	43	91	446	579 ± 43
4	4	54	70	270	394 ± 26
4A	4	46	66	349	461 ± 12
5	3	62	121	479	662 ± 121
6	5	78	134	628	840 ± 69
6A	5	99	161	686	946 ± 83
7	2	36	56	210	302 ± 39
8	5	47	53	377	477 ± 42

No method utilizing acetonitrile approached the efficiency of hexane:ethanol for extracting these compounds from green alfalfa. Method 5 which included a 16-hour delay between extraction and filtering yielded the highest residue of the acetonitrile combinations. Soxhleting alone, method 2, gave very low results.

Table 2 presents the percentage of the total extraction method contributed by Soxhleting following the initial extraction by the most exhaustive method, 1A. It would appear from these

results that the additional 4.6% mean residue obtained through the 16 hours of reflux are only of academic interest and would be of little value to commercial residue laboratories.

TABLE 2

Percentage of total residue recovered after 16 hours Soxhleting of plant material with methanol:chloroform (1:1) following initial extraction by the most efficient method (1a).

SAMPLE	DDE	o,p DDT	p,p' DDT	TOTAL
2	11.0%	5.1%	2.0%	3.4%
3	3.4%	1.8%	2.3%	2.3%
4	10.3%	5.6%	9.6%	9.0%
5	3.8%	2.3%	4.3%	3.9%
Mean %	7.1%	3.7%	4.6%	4.6%

Conclusions

1. Hexane:ethanol (2:1) at a ratio of 6 ml per gram of green alfalfa is the superior solvent mixture for extracting DDT and related materials.
2. Allowing the plant material to stand in the solvent 16 hours after mincing is the most thorough extraction method.
3. Eluting the Florisil column with 20% methylene chloride in petroleum ether increases total residue recovery by 18%.

4. Exhaustive Soxhleting with methanol:chloroform of the plant material after the initial extraction yields only an additional 4.6% residue and probably is of no practical value to commercial residue laboratories.

References

1. P. F. BERTUZZI, Private communication Food and Drug Admin., U. S. Dept. of Health, Education and Welfare (1967)
2. R. O. MUMMA, W. B. WHEELER, D. E. H. FREAR, and R. H. HAMILTON, Science 152, 530 (1966)
3. G. A. ROOT, Bull. Environ. Contam. and Toxicol. 2, 274 (1967)
4. W. B. WHEELER, D. E. H. FREAR, R. O. MUMMA, R. H. HAMILTON and R. C. COTNER, J. Agr. Food Chem. 15, 227 (1967)
5. F. M. WHITING, J. W. STULL, W. H. BROWN, MARY MILBRATH and G. W. WARE, J. Dairy Sci. 51, 1039 (1968)