Differences in DDTR Residues in Green Alfalfa, Hay, and Cubes from the Same Source[1]

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During the period 1964-68, when DDT use in Arizona was declining because of high residues found in forage, alfalfa hay buyers and growers alike were having residue analyses conducted on their products by commercial laboratories. In the process some of the more curious alfalfa growers submitted 2 samples for analysis, one hay and the other hay cubes collected at the same time from the same field. The analytical results usually indicated substantial differences between the two. Consequently we have frequently been asked, why the difference?

It was the purpose of this investigation to determine whether the analytical results of inherent DDTR in various forms of alfalfa collected at the same field and time were in fact different.

Four agricultural areas in Arizona were selected for alfalfa hay sampling because of their varying use rate of DDT--Safford, low, and Higley, Casa Grande and Coolidge, high. The first 3 samples were collected in mid-August 1970, 20 months after the onset of the agricultural DDT moratorium, and the latter in May, 1971.

In each of the four fields, samples were collected during the actual cubing of field-dried, windrowed alfalfa hay. Four 20-cube samples were removed from the bin of the cubing machine (ca. 3 lbs); four windrow hay samples were collected around the machine (ca. 1 lb), and four green standing alfalfa samples were collected from scattered clumps between the windrows, field edges and irrigation borders (ca. 3 lbs). All samples were placed in large polyethylene bags, the green alfalfa being held on ice.

Sub-samples of green alfalfa were dried with the aid of incandescent light 7 days in the laboratory for dry-weight determination and for lab-dried hay analysis.

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The remaining green alfalfa was macerated in a Hobart salad chopper and frozen. The laboratory hay, windrow hay and hay cubes were finely ground in a Wiley mill and stored at room temperature.

All samples were replicated three times. The green alfalfa samples were extracted as in earlier work (1), 50 grams in 300 ml of 2:1 hexane:ethanol stirred with an omnimixer for 10 minutes, and allowed to stand overnight in the refrigerator at 4°C. 20-gram samples of the powdered cubes and hays were extracted with 200 ml of chloroform:methanol, 1:1 (2), for 10 minutes on the omnimixer and allowed to stand at room temperature for 5 hours. The 1971 cubes and hay were extracted with 300 ml of 2:1, chloroform:methanol.

After washing and removal of the alcohols, aliquots of the green alfalfa extracts were eluted from a 4-in. Florisil column with 200 ml of 6% ethyl ether in hexane. For the cube and hay extract cleanups the eluant was 5% ethyl ether in hexane.

All extracts were then dehydrochlorinated (3) with ethanolic NaOH to produce DDE, washed, and dried by passing through Na_2SO_4. The hexane samples were concentrated on a steam bath and transferred to glass stoppered 12 ml centrifuge tubes.

Analysis for p,p'- and o,p'-DDE was by ECGC. The chromatograph conditions for analysis involved an 8', 4 mm I.D. Pyrex glass column, containing 100-120 mesh Chromosorb-W treated with 4% SE 30 and 6% QF-1. Preceding the packing was a 1" plug of anhydrous cadmium chloride followed by a 1" plug of sodium carbonate (3). Gas flow was 79 ml/min and operating temperatures were 223°, 215° and 228°C, for the injection port, column, and detector, respectively. Replicate GC analyses were conducted daily to confirm accuracy.

Standards carried through the extraction and dehydrochlorination systems gave 80% recovery for o,p'-DDE and 97% for p,p'-DDE. ECGC retention times were 5.75 min for o,p'-DDE and 7.12 min for p,p'-DDE.

The data presented in Table 1 indicate that in the first 3 fields, collected in August, the DDTR residues were: cubes > field hay > lab hay > green alfalfa, expressed either as wet or dry weight.

These results were unexpected and another sampling was conducted in a Coolidge field the following spring, with the results (Table 1): cubes > green alfalfa > lab hay > field hay.

The residues observed in the cubes in all 4 studies were generally 63% higher than those found in the windrow hay from which the cubes were derived. Theoretically all values within a sampling should have been the same. The methods used for extraction and cleanup with all hay forms were those proven to be

TABLE 1

DDTR residues expressed as PPB DDE in 4 forms of alfalfa from the same fields collected in 4 agricultural areas of Arizona.

	Dry Weight			Wet Weight[1]		
	o,p'-	p,p'-	Total	o,p'-	p,p'-	Total
SAFFORD						
Cubes	<8	72	80	<3	20	23
Field Hay	<8	42	50	<3	12	15
Lab Hay[2]	--	--	--	--	--	--
Green Alfalfa	<8[3]	35[3]	43	<3	10	13
HIGLEY						
Cubes	37	400	433	9	105	113
Field Hay	29	304	333	8	80	87
Lab Hay	24	214	238	7	56	62
Green Alfalfa	<8[3]	154[3]	162	<3	40	43
CASA GRANDE						
Cubes	38	346	384	15	135	150
Field Hay	21	170	191	8	67	75
Lab Hay	19	122	141	7	48	55
Green Alfalfa	<8[3]	73[3]	81	<5	29	34
COOLIDGE						
Cubes	33	383	416	9	108	117
Field Hay	27	237	264	8	67	75
Lab Hay	23	292	315	7	82	89
Green Alfalfa	27[3]	313[3]	340	8	88	95
Cubes, OD[4]	35	396	431	10	111	121
Field Hay, OD	26	258	284	7	73	80
Lab Hay, OD	27	271	298	8	76	84

1. Residue in equivalent weight of green alfalfa.

2. Sample lost—moisture content estimated.

3. Values determined only by extrapolation from wet weight residues.

4. Oven dried at 80°C for 24 hrs.

the most exhaustive in this laboratory and are the ones used to report the DDT moratorium monitoring data.

The discrepancies appear to be related to three factors: (a) moisture content of the product at extraction, (b) rate of drying from green to dried form, and (c) cellular disruption.

Several studies have been conducted by other workers indicating that with dry substrates, moisture content is important, depending on the solvent combination, when total organochlorine insecticide residues are extracted. In our studies the rate-of-drying factor is only moderately supported, in field and lab, but indicates that residue levels and drying rate are directly related.

Cellular disruption may involve several facets. First, we have observed that by powdering hay samples on a Wiley mill prior to extraction, residue levels increased from 30% to 60%, over chopped hay. Root (4) has made similar observations. Secondly, hay cubing machines currently used compress hay under pressures ranging from 6,000 to 10,000 p.s.i., producing cubes having a bulk density of 25 to 30 lbs/ft^3. During cubing the hay is sprayed with water to facilitate the operation, and compressed to a density approximately 4 to 5X that of baled hay. From this sudden heat increase the alfalfa lignen with the water acts as an adhesive.

The cubing process, then, must alter the residue status in dry alfalfa, either through the sudden heat increase, resulting from the friction of compression, or cellular disruption, making the cell contents more accessible to the solvent.

This preliminary investigation indicates the need for a more detailed study into the type of association DDTR residues have with plant tissues when infinitesimal residues are a part of the plant's growing environment.

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