A Rapid Analytical Method for Persistent Pesticides in Proteinaceous Samples

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A recent investigation (1,2) of the relationship between dietary intake of pesticides and subsequent tissue levels required development of a very rapid, simple, and highly sensitive method for the detection and estimation of chlorinated hydrocarbon insecticides in proteinaceous materials. The following procedure has been applied successfully to about 3000 samples of this type in our laboratory and has been particularly useful for analysis of DDT and its relatives in small samples of bovine milk and blood.

Experimental

Chemicals and Equipment. All chemicals were reagent grade. Pesticides were analytical standards supplied by the manufacturer or purified in our own laboratory, and the reagent grade solvents were redistilled shortly before use. The gas chromatograph was an Aerograph Model 600B (Wilkens Instrument Co.) equipped with an electron capture detector and a Leeds and Northrop Model G 1 mv. recorder. The most satisfactory chromatographic column was a 9' x 1/8" stainless steel tube packed with 60/80 mesh HMDS-treated Chromosorb W containing 5 per cent Dow 710 silicone oil and 5 per cent SE-30 gum rubber. The 12" section of the column nearest to the injection port was packed with 20/30 mesh calcium carbide for removal of traces of water and ethanol. Nitrogen carrier gas (40 p.s.i. 60-100 ml./min.), a column temperature of 240-250°C, and an instrument attenuation of 1X gave best results.

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A recorder chart speed of 60"/hr. was convenient, and the peak areas were measured with a Polar planimeter.

The dehydrohalogenation reagent was prepared fresh daily by dissolving (for each sample) 5 g. of C.P. potassium hydroxide in 3 ml. of distilled water and adding, with stirring, 17 ml. of ethanol.

Analysis of Milk. The milk was warmed to 40°C., mixed well, and a subsample (generally 10 ml.) was pipetted into a glass-stoppered Pyrex test tube (about 50 ml. capacity) containing 20 ml. of the KOH reagent. The tube was stoppered, shaken, heated in a water bath at 75-80°C. for 15 min., cooled, and 10 ml. of pentane was added to its contents. After shaking for 4 min., the layers were allowed to separate, and an aliquot of the upper (hydrocarbon) layer was withdrawn with a syringe and injected directly into the chromatograph. If the pentane and alkali phases did not separate immediately, the addition of 10 ml. of distilled water hastened the separation. In this instance, one µl. of the pentane solution was equivalent to 1 mg. of milk. The amount of pesticide in the aliquot was determined by comparing the area under the peak on the strip-chart to those obtained with appropriate dehydrohalogenated standard solutions (containing 0.5 ng. in the case of DDT).

Analysis of Blood. Citrated, oxalated, or heparinized blood (0.1 - 1.0 ml.), thoroughly mixed, was introduced into 2 ml. of the KOH reagent and then analyzed according to the procedure for milk with the exception that only 1 ml. of pentane was added for extraction.

Analysis of Fat and Flesh. The sample (1-2 g.) was weighed into the 5 ml. cup of a VirTis micro homogenizer and blended with 5 ml. of pentane. The homogenate was transferred to a 500 ml. boiling flask with about 200 ml. of an equivolume mixture of pentane and ether, boiled under reflux for 1 hr., cooled, filtered, and the solvent removed in vacuo on a rotating evaporator. The residual extractives were weighed, 10 ml. of pentane added, and

an aliquot equivalent to 0.5 g. of extractives was transferred to a glass-stoppered test tube. The solvent was removed in a stream of air, and 20 ml. of KOH reagent was added. Analysis then was conducted according to the milk procedure.

Alternatively in each of these analyses, a round-bottomed flask could be substituted for the test tube, and the alkaline dehydrohalogenated mixture could be transferred to a separatory funnel for pentane extraction. This was useful when relatively large samples were to be analyzed.

Results and Discussion

As shown in Table 1, a number of chlorinated hydrocarbon insecticides may be estimated in proteinaceous samples by this "dehydrochlorination" procedure. Toxaphene, heptachlor, and endrin provided well-resolved, multiple peaks, one of which predominated. Although a few almost coincident peaks occurred, those of an intensity greater than 5 per cent of the DDT peak were distinctly separated.

TABLE 1.

Retention Times of Dehydrochlorinated Pesticides

Compound	Retention Time in Minutes (Peak Intensity[a])		Compound	Retention Time in Minutes (Peak Intensity[a])	
Lindane	0.4	(13.9)	DDD (p,p')	6.7	(64.0)
Toxaphene I	2.7	(0.4)	Endrin II[b]	7.2	(1.1)
Toxaphene II	3.2	(0.4)	Toxaphene V	8.1	(1.0)
Heptachlor I[b]	4.1	(7.3)	DDT (p,p')	8.3	(100.0)
Aldrin	4.6	(97.3)	DDE (p,p')	8.3	(100.0)
Toxaphene III[b]	4.8	(2.4)	Endrin III	9.7	(0.5)
Heptachlor II	5.1	(3.1)	Dieldrin	10.3	(5.8)
Endrin I	5.2	(0.5)	Heptachlor epoxide[c]		0.0
Toxaphene IV	6.7	(0.9)	Thiodan[c]		0.0

[a] DDE 100, [b] Principal peak, [c] no peak at 30 ng.

The major part of our work involved measurement of total DDT and relatives (p,p'-DDT, o,p'-DDT, p,p'-DDE, and DDD) in bovine milk, bovine and human blood, poultry eggs, and the fat/flesh from cattle, deer, geese, pheasants, and fish. Extracts of plant products including alfalfa hay, feed formulations, and pasture grass generally required the addition of a Florisil cleanup step to minimize pigments. DDT could not be distinguished from DDE by this method, of course, and the retention times of o,p'-DDE and the dehydrohalogenation product of p,p'-DDD were coincident: analysis for the total DDT group therefore was greatly simplified. The very high column temperature gave convenient retention times, sharp peaks, and excellent column life without detector fouling. A single column functions well for more than a year, and detector sensitivity was not affected when the column was well conditioned.

Analytical sensitivity and accuracy were very satisfactory. Standard recoveries were essentially quantitative, and, in the ranges most frequently encountered, results were reliable to within ± 3 per cent. (Table 2). Other experiments showed that the

TABLE 2

Probable Error (P) in Analyses of Several Sample Types

Sample	No. of Samples	DDT[a] (p.p.m. ± P)	± P (Per cent)
Whole milk	5	0.645 ± 0.006	0.9
Colostrum	5	0.0031 ± 0.00003	1.0
Bovine Blood	5	0.408 ± 0.0009	2.2
Bovine Blood	5	0.00302 ± 0.0004	1.2
Bovine Blood	5	0.00061 ± 0.00002	2.9

[a] Total of DDT and related compounds

error was almost equally divided between the sampling-extraction-cleanup steps and the chromatography-measurement steps. During our investigation, Schafer, et al (3) described a very similar procedure in which peak height was used for quantitation; we found, however, that measurement of peak areas at these very low levels offered a notable increase in accuracy over the other method.

The principal advantages of this method lie in (a) a degree of simplicity which permits a large volume of high-protein and/or high-fat samples to be handled routinely, (b) the very effective cleanup afforded by the hot alkali, and (c) the chemical conversion of a variety of "persistent" pesticides into derivatives more readily separated and detected than the parent compounds. We are indebted to R. C. Laben and S. A. Peoples for milk, blood, and tissue samples; to H. F. Beckman for helpful discussions; to Nels Larsen and Eugene Whitehead for technical assistance; and to Margaret Schafer for a prepublication copy of her excellent manuscript.

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