Determination of 2,4-D Residues in Animal Products

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Several simplified methods for the analysis of pesticide residues in animal products have been reported recently from our Laboratory (1). The present communication is concerned with a rapid and convenient method for cleanup and analysis of high protein samples for the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D).

Marquardt and Luce (2) applied a colorimetric analysis to 2,4-D in milk, and Coakley, et al. (3), used the same procedure for determination of the acid and its esters in shellfish and fish. Burchfield and Storrs (4) and Marquardt, Burchfield, and Storrs (5) have described a method for milk analysis based on microcoulometric gas chromatography using internal standards. In each of these instances, the isolation of 2,4-D in a form suitable for analysis proved to be rather complicated, and each analytical method presented serious special problems. The procedure described here, in which milk was employed as a typical high-protein substrate, proved to be simple, sensitive, and relatively inexpensive.

Experimental

Chemicals and Equipment. All chemicals were reagent grade, 2,4-D was a recrystallized analytical standard, and the reagent grade solvents were redistilled shortly before use. Diazomethane in ether was prepared from "Diazald" according to directions of the manufacturer (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin). The gas chromatograph was an Aerograph Model 600B (Wilkens Instrument Co.) equipped with an electron capture detector. A 5' x 1/8" stainless steel column packed with either 5% Dow 11 silicone oil or 5% SE-30 gum rubber on 60/80 mesh Chromosorb W was satisfactory. A column temperature

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of 197°C, injection port temperature of 240°C, and nitrogen carrier gas flow of 50 ml./min. at 14 p.s.i. were maintained.

Cleanup. A sample of milk was warmed to 40°C, mixed well, and a 25 ml. subsample was transferred to a 250 ml. centrifuge bottle. Petroleum ether (25 ml.), diethyl ether (25 ml.), and 1 N aqueous sodium hydroxide solution (1 ml.) were added, the stoppered bottle was shaken mechanically for 30 min. to extract fats and other neutral substances, and the layers were caused to separate by centrifugation for 15 min. at 1700 r.p.m. The upper layer was decanted, and the ether-petroleum ether extraction was repeated. The aqueous phase then was transferred to a 300 ml. flask fitted with a reflux condenser, and 25 ml. of methanol and 2 g. of sodium hydroxide were added, the solution was boiled for one hour, cooled, acidified with 250 ml. of 0.5 N hydrochloric acid, and the 2,4-D was extracted into three successive 15 ml. portions of chloroform. At this point, the extract may be pure enough for analysis; if not, it may be washed with two 25 ml. portions of 1% aqueous sodium bicarbonate solution, the combined bicarbonate washes acidified, and the released 2,4-D reextracted into chloroform.

Analysis. An aliquot (40%) of the chloroform extract was evaporated under a stream of air, 1 ml. of an ether solution of diazomethane was added, and, after about 5 min., the solution again was evaporated. Hexane (1.0 ml.) and 6% aqueous sodium sulfate solution (5 ml.) were added, and an aliquot was subjected to gas chromatography. One microliter of the hexane solution represented 10 mg. of milk and contained one ng. of 2,4-D (as the methyl ester) if the original milk level was 0.1 p.p.m.

Results and Discussion

As shown in Table 1, recoveries of 2,4-D standards were approximately 90%, and the practical limit of detection was 0.5 ng. in a sample representing 10 mg. of milk (0.05 p.p.m.). Retention time of 2,4-D methyl ester on the SE-30 column was 5 min.; under

the same conditions, aldrin and DDE had retention times of 15 min. and 40 min., respectively, so that the desired peak was well-separated from other common pesticide contaminants.

TABLE 1

Recovery of 2,4-D From Milk

Concentration Calculated (p.p.m.)	Concentration Found (p.p.m.)	Quantity Found (ng.)	Recovery %
0	0	0	-
0.050	0.042	0.42	84
0.10	0.093	0.93	93
0.20	0.18	1.80	90

Although intended primarily for the estimation of 2,4-D, this procedure should apply equally well for the analysis of other phenoxy herbicides such as 2,4,5-T, 2,4-DB, Silvex, and MCPA; halogenated phenylacetic acids such as Fenac; and other halogenated acids including trichlorobenzoic acid and Dicamba. The procedure is suitable for the free acids and their salts; esters and amides, removed in the initial ether-petroleum ether extraction, undoubtedly would require much more detailed clean-up before they could be analyzed in this way.

It has long been observed that high-protein samples appear to bind 2,4-D, and the methods developed to avoid this difficulty have become quite elaborate. Recoveries may be low, and, in our experience, high electron-capture backgrounds may be encountered. The present method conveniently and effectively avoids these problems and appears to offer general application to blood, meat, and many other animal products.

References

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