DDT, Aldrin, and Dieldrin Residue Determinations in Milk and Butter

by R. MESTRES, F. BARTHES, and M. PRIU
Faculté de Pharmacie
Université de Montpellier, France

The constant search of pesticide residue chemists for a better and faster approach to the organochlorine pesticide content of dairy products has long offered to official regulatory agencies the means to control this problem as shown by the relationships (2, 3, 4) among the thousands of analyses already made.

Our purpose is not to analyze the various adaptations of the different steps of the general and well-known MILLS' method that we have adapted to our equipment and to our conception of the analysis, but rather to present our latest refinements (8) for the determination of DDT, aldrin, and dieldrin in milk and butter.

PRINCIPLES

A moderate amount of milk or butter is submitted to an alkaline hydrolysis prior to extraction by petroleum ether. The extract is then purified on a Florisil column in such a way that the resulting DDE and aldrin are eluted selectively by petroleum ether alone. Dieldrin is eluted later with a mixture of diethyl ether----petroleum ether.

Determinations are made on the concentrated purified extract by electron-capture or microcoulometric gas chromatography. The sensitivity of the method is voluntarily limited for routine

Bulletin of Environmental Contamination & Toxicology,
Vol. 2, No. 1, 1967, published by Springer-Verlag New York Inc.

determinations at 0.01 p.p.m. for aldrin and 0.02 p.p.m. for DDT and dieldrin in milk and quantities ten times higher in butter. An infrared method is used to confirm the presence of residues of DDT in milk higher than 0.5 p.p.m.

EXPERIMENTAL

Chemicals:

a) Petroleum ether (P.E.) b.p. 45-60° C., chemically purified and redistilled.

b) Water: wash freshly distilled water with P.E. reagent a.

c) Sodium oxalate, 5% in water b.

d) Potassium hydroxide, 2N: dissolve 15 g. in 50 ml. of water b and add 100 ml. of 95% ethanol; wash this solution with 30 ml. of a.

e) Sodium chloride, sodium sulfate, and Florisil: heat for 48 hours to at least 180° C. before use; store at 130° C.

f) Florisil: before use, weigh 100 g. of preheated Florisil, wet it with 5 ml. of water, shake it thoroughly, and let it stand overnight in a closed flask at room temperature; use it within 3 days.

g) Saturated sodium chloride solution in b.

h) Sulfuric acid, dilute 1:9 and wash with a.

i) Eluting solvents: (1) 100% a and (2) 20 % diethyl ether in a.

j) Standard solutions: DDE 1 p.p.m., aldrin 0.25 p.p.m., and dieldrin 0.5 p.p.m. in a.

k) Paraffin oil, 1% in a.

1) Carbon disulfide, spectrophotometrically pure.

Special glassware:

a) Hydrolysis apparatus: 100 ml. borosilicate r.b. flask attached to a vertical condenser with a Teflon-coated $\frac{T}{S}$ 24 joint.

b) Separatory funnels, 125 and 250 ml. with Teflon stopcocks and glass stoppers with Teflon sleeves.

PROCEDURE FOR MILK

Hydrolysis:

Place 5 ml. of milk, 1 ml. of c, and 20 ml. of d in the 100-ml. flask, attach the condenser, and boil one hour.

Extraction:

Transfer this cooled soap solution to a 125-ml. separatory funnel containing 10 ml. of g. Wash the flask with 25 ml. of a and collect the washings in the funnel. Shake thoroughly; after separation drain the aqueous phase into a 100-ml. beaker and pour the ether phase into another 100-ml. beaker. Transfer the aqueous phase back into the funnel, again wash the hydrolysis flask with 25 ml. of a and add the washings to the funnel; shake moderately. After separation, discard the aqueous phase. Combine the two ether phases and wash once with 5 ml. of h, and three times with 10 ml. of b. Dry the ether extract with sodium sulphate e. Concentrate it under a jet of filtered air, in the water bath, to ca. 10 ml.

Florisil column chromatography:

Prepare the column (2 cm. I.D.) with 2 inches of f between two 1/2-inch layers of sodium sulphate e. Prewash the column with 40 ml. of i (2) and then 50 ml. of a. Transfer the concentrated extract to the top of the column. Rinse the beaker with a few ml. of a and elute successively with 100 ml. of a and 40 ml. of i (2), collecting the two eluates in separate beakers.

PROCEDURE FOR BUTTER

Hydrolysis:

Use 0.5 g. of butter and 10 ml. of d.

Extraction:

Transfer the cooled soap solution to a separatory funnel containing 4 ml. of b and 6 ml. of g. Wash the flask with 25 ml. of a and proceed according to the procedure for milk.

DETERMINATION OF RESIDUES

Electron-capture gas chromatography:

1) Aldrin and DDE

The first eluate quantitatively isolates aldrin and DDE. Add 0.5 ml. of k as a keeper and concentrate to 2 ml. under vacuum in a rotating evaporator. Gas chromatographic analysis is performed as usual with both DC 200 and QF 1 columns; the injection is limited to 2 µl. of the concentrated extract, equivalent to 5 µl. of milk or 0.5 mg. of butter.

If aldrin or DDE appears on the two columns, areas are compared with the areas of standards under the same operating conditions. Areas are by integration or by product of height of

the peak times width at 54.6% of the height, measured from the top (5). Peaks less than 10% of scale (less than 0.1 ng. of DDE and 0.05 ng. of aldrin) are ignored. These restrictions limit the sensitivity to 0.02 p.p.m. of DDT, and 0.01 p.p.m. of aldrin in milk and quantities ten times greater in butter; results equal to or less than these limits are expressed as 0.00 p.p.m. in milk and 0.0 p.p.m. in butter.

2) Dieldrin

Dieldrin is quantitatively recovered in the second eluate. Add 0.5 ml. of k and evaporate to 2 milliliters on the water bath under a jet of filtered air. Inject 2 µl. and proceed as for aldrin and DDE. Peaks below 10% full scale are ignored (less than 0.1 ng. of dieldrin, which limits the sensitivity to 0.02 p.p.m. of dieldrin in milk and 0.2 p.p.m. in butter).

3) General

For routine butter analysis we assume these sensitivities as sufficient. They can be lowered five to ten times with an extract concentrated to 0.5 ml. Any positive result will then only be considered as the lowest limit above which the absence of residue may be acknowledged.

Recovery tests have shown complete recovery for DDT (as DDE), aldrin, and dieldrin introduced into milk or butter before the hydrolysis step; fortified samples contained, respectively, 0.2 p.p.m. of DDT, 0.05 p.p.m. of aldrin, and 0.10 p.p.m. of dieldrin in milk and 2.0, 0.5, and 1.0 p.p.m. in butter.

QUALITATIVE AND QUANTITATIVE VERIFICATIONS

Relative retention times:

The first step in the characterization of observed peaks is the measure of their relative retention times t_{RR} versus standard aldrin on the two columns. Comparisons among unknowns and standards are made during the same working period.

Thin-layer chromatography:

Remaining extracts are spotted on the thin-layer plate and analyzed according to known techniques (e.g., 1, 6, 7). The average sensitivity of 1 μg. per revealed spot allows the determination of 0.2 p.p.m. of residue in milk and 2.0 p.p.m. in butter.

Microcoulometric gas chromatography:

Concentrate the extract remaining after electron-capture glc to 0.1 ml. and inject 25 μl. (equivalent to 1.25 ml. of milk or 0.125 g. of butter) into the chromatograph. The clear response of the Dohrmann C 200 microcoulometer for 10 ng. of aldrin or dieldrin and 40 ng. of DDE allows their easy determination in milk above 0.01, 0.01, and 0.04 p.p.m., respectively, and in butter above 0.1, 0.1, and 0.4 p.p.m.

Infrared determination of DDT in milk:

Sensitivities achievable by infrared spectrophotometry are far inferior to the above glc techniques and much larger initial material is required. Following the same principle as in the foregoing method, the infrared determination of DDT in milk can be achieved for residues above 1 p.p.m., with a lower limit of detectability of ca. 0.5 p.p.m.

1) Equipment

Perkin Elmer InfraCord 237 with sodium chloride cavity cells 5 mm. thick.

2) Procedure

Introduce into the 100-ml. flask 45 ml. of milk, 4 ml. of c, and 30 ml. of d. Attach the condenser and boil one hour. Let cool, transfer to a 250-ml. separatory funnel, add 2 g. of sodium chloride e and extract twice with 30 ml. of a. Collect the ether phases in the flask and evaporate to 15-20 ml. on a steam bath under a jet of filtered air. Add 20 ml. of d and again saponify for one hour. Transfer the soap solution to a separatory funnel with 4 ml. of b, add 3 ml. of g, and extract twice with 30 ml. of a. Wash the combined ether phases with h and water as in the general method. Dry with sodium sulphate e, evaporatively concentrate to ca. 5 ml. and chromatograph onto a Florisil column prepared as before. Elute with 100 ml. of a. Evaporate just to dryness without addition of paraffin oil, dissolve the dry residue in 0.5 ml. of l and with this solution fill the 5-mm. cavity cell. Scan from 1,300 to 625 $cm.^{-1}$, with a similar cavity cell with solvent only in the reference beam.

DDE is identified by its bands at 1,090, 1,015, 970, and 784 $cm.^{-1}$. If a spectrum is not clear enough, draw the extract from the cavity cell into a syringe and transfer it to a new Florisil column. Rinse the cell with l and add the washings to the column. Elute again with 100 ml. of a, evaporate the new eluate, and repeat as before.

Quantitative estimations are by means of the base-line method for bands at 1,015 or 970 cm.$^{-1}$. With our equipment we have found, for instance, $c = 1.5 \cdot A_{1,015}$ and $c = 3.7 \cdot A_{970}$, where c = DDE in g./l. of carbon disulfide. The recovery from fortified samples averages 75%.

RESULTS

Among 20 samples of milk analyzed by this method, one sample showed a high residue of 0.25 p.p.m. of DDT by both types of glc; the attempt to confirm it by infrared spectrophotometry was not made. Forty-four samples of butter in France and 4 in Switzerland showed no apparent residues of DDT, aldrin, or dieldrin, assuming that any residues in these samples cannot be higher than 0.2 p.p.m. of DDT, 0.1 p.p.m. of aldrin, and 0.2 p.p.m. of dieldrin. Further examination of the concentrated extracts of these samples showed that the limits of possible residue contents were at least five times lower; as the chances for errors increase when a detectability limit is stretched, however, we assume that the first figures are sufficient for a fast and accurate routine method.

ACKNOWLEDGMENT

We acknowledge with thanks the cooperation of Mr. E. PORTAL, Director of the Laboratoire de la Répression des fraudes of Montpellier who provided most of the samples. This work was aided by I.N.S.E.R.M. research funds (C.R. Nutrition 66-425).

REFERENCES

(1) R. A. CONKIN, Residue Reviews 6, 136 (1964).

(2) D. G. CROSBY and T. E. ARCHER, Bull. Environmental Contamination and Toxicol. 1, 16 (1966).

(3) W. F. DURHAM, Residue Reviews 4, 60 ff. (1963).

(4) J. L. HENDERSON, Residue Reviews 8, 74 (1965).

(5) P. JAULMES and R. MESTRES, Chim. Phys. p. 929 (1959).

(6) M. F. KOVACS, J. Assoc. Official Agr. Chemists 48, 1018 (1965).

(7) R. MESTRES and F. BARTHES, O.I.V.V., Feuillets Pest. No. 8 (1965).

(8) R. MESTRES, F. BARTHES, M. PRIU, and E. PORTAL, Trav. Soc. Pharm. Montpellier, 26, in press (1966).