A Screening Method for Chlorinated Pesticide Residues in Fat Without Cleanup

LEO F. KRZEMINSKI and
WENDELL A. LANDMANN
Division of Analytical and Physical Chemistry, American Meat Institute Foundation, Chicago, Ill.

A screening method for the estimation of total chlorinated hydrocarbon residues in fat has been developed. The method can be used on fat samples without the isolation of the pesticide residue. Fat samples, from which water-soluble inorganic chloride ion has been removed by heat rendering adipose tissue through filter paper, are treated with sodium in anhydrous liquid ammonia to reduce organically bound chlorine to chloride ion. Reduction is complete in a few minutes and gives better than 90% recovery for a 10-gram fat sample containing 10 p.p.m. DDT. Chloride ion concentration is measured potentiometrically and converted to p.p.m. insecticide by referring to a standard calibration curve.

THE CHLORINATED HYDROCARBON PESTICIDES are most likely to be found as residues in adipose tissue because of their high fat solubility, slow metabolism, and slow excretion from the animal body. To detect this class of pesticides chromatographic techniques are generally satisfactory, but may require facilities not readily available in all laboratories. Several procedures have been described for rapid estimation of chlorinated hydrocarbon residues by determining total chlorine in the sample (*5, 7, 8*). However, most of these procedures require isolation of the hydrocarbon in varying degrees of purity prior to analysis. Because of the time-consuming isolation (cleanup) step in animal fats, results may be delayed beyond their usefulness. If a screening procedure is to be as rapid and simple as possible, the isolation step should be eliminated. It seemed likely that sodium in liquid ammonia could be used to reduce microgram quantities of pesticides in the presence of gram quantities of fat.

The use of sodium and ammonia mixture for the reduction of chlorinated compounds was first reported by Chablay (*3*), and was applied to the determination of halides in insecticides by Beckman (*1*). This method was investigated with regard to the determination of organically bound chlorine in the presence of a large excess of fat, thereby eliminating the usual cleanup step. Measurement of the chloride ion concentration was performed by a modification of the potentiometric method described by Furman and Low (*4*). Experiments were conducted with DDT as the model compound. Since Beckman has already shown that the sodium–liquid ammonia system is capable of quantitatively reducing pesticides of this type, this method should be applicable to pesticides other than DDT.

Experimental

Apparatus. Potentiometer, Leeds & Northrup Hydrogen Ion Potentiometer, No. 7655, or a similar type with a sensitivity of ±0.0003 volt.

Graduated oil tube (100 ml. ASTM conical form with stem graduated from 1 to 3 ml.).

Pipet (1.0-ml., graduated in 0.01 ml., with tip cut off to facilitate transfer of sodium dispersion).

Reagents. Anhydrous liquid ammonia (Armour, refrigeration grade, 99.99%).

Dispersed sodium (Anderson Chemical Co.), 50% dispersion in xylene, diluted with xylene to 10% dispersion.

Methyl Cellosolve solution, 50% aqueous solution of ethylene glycol monomethyl ether containing 5% concentrated nitric acid.

Salt solution (0.8654 gram of sodium chloride per 100 ml. of water).

Preparation of Silver–Silver Chloride Electrodes. Clean two 10-inch pieces of silver wire (20 gage) with ethyl alcohol, ethyl ether, and detergent; rinse with water. Bend each silver wire into a helix by wrapping it around a glass rod $^1/_8$ inch in diameter. Prepare a 5% sodium chloride solution and add approximately 1 ml. of concentrated hydrochloric acid per 100 ml. of solution. Place the two helices of silver wire into a 250-ml. beaker of salt solution and connect them to the negative pole of a 1.5-volt dry cell battery. Connect another piece of silver wire to the positive pole of the battery. Allow the current to flow through the circuit until the pair of helices appear clean; then reverse the connections and electrolyze the silver helices for 15 minutes. Store the pair of electrodes in water when not in use. Use cork stoppers, slit to provide venting of trapped air, to support the electrodes in the concentration cell.

Handling of Liquid Ammonia. Use a hood with a strong draft and exclude moisture from the reaction mixture as much as possible. Wash and thoroughly dry a 1-quart, narrow-neck Dewar bottle. Invert a cylinder of liquid ammonia (in the case of a large cylinder, elevate the bottom) and place the Dewar flask under the orifice of the valve. Wrap Parafilm or plastic sheeting loosely around the valve and the mouth of the Dewar to prevent moisture from condensing on the cold parts. Fill and stopper the Dewar with a loosely fitting cork. Measure the ammonia for the determination with a clean dry graduated cylinder. A needle valve attached to an extension from the tank may be used for direct delivery of liquid ammonia to a graduated cylinder (*1*).

Concentration Cell. Construct the cell as shown in Figure 1.

Procedure

Rendering the Sample. Macerate 20 to 30 grams of diced adipose tissue (approximately 1-cm. cubes) with fine quartz sand in a mortar. Transfer the sample and sand into a circle of fluted filter paper (Whatman No. 12) fitted to a glass funnel supported by a beaker and place into a hot air oven at 120° C., Allow the sample to remain in the oven for approximately 30 minutes. Weigh 10 grams of the heat rendered fat into a 125-ml., glass-stoppered Erlenmeyer flask.

Sodium Reduction. Dissolve the fat in 20 ml. of anhydrous ethyl ether. To this solution, add 0.5 ml. of a 10% mixture of metallic sodium in xylene, and cool by swirling in a liquid ammonia bath (−33° C.) until the fat precipitates out of solution as a slush. Do not allow the mixture to solidify. To the cold slushy mass, add 100 ml. of anhydrous liquid ammonia, swirl, and allow the ammonia to evaporate. Remove the last traces of ammonia and ether by warming gently on the steam bath or by allowing the mixture to stand overnight. To the dry residue left in the Erlenmeyer flask, add 100 ml. of Skellysolve B (redistilled) and 10.0 ml. of methyl Cellosolve solution. Stir the phases with a glass rod until complete solution of the residue

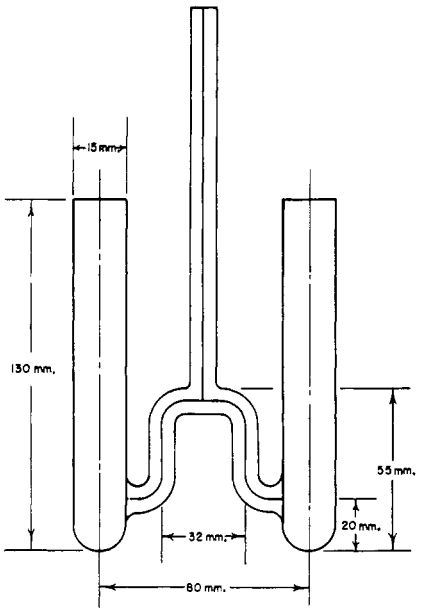

Figure 1. Concentration cell constructed from 2-mm. capillary tubing and two culture tubes (130 × 15 mm.)

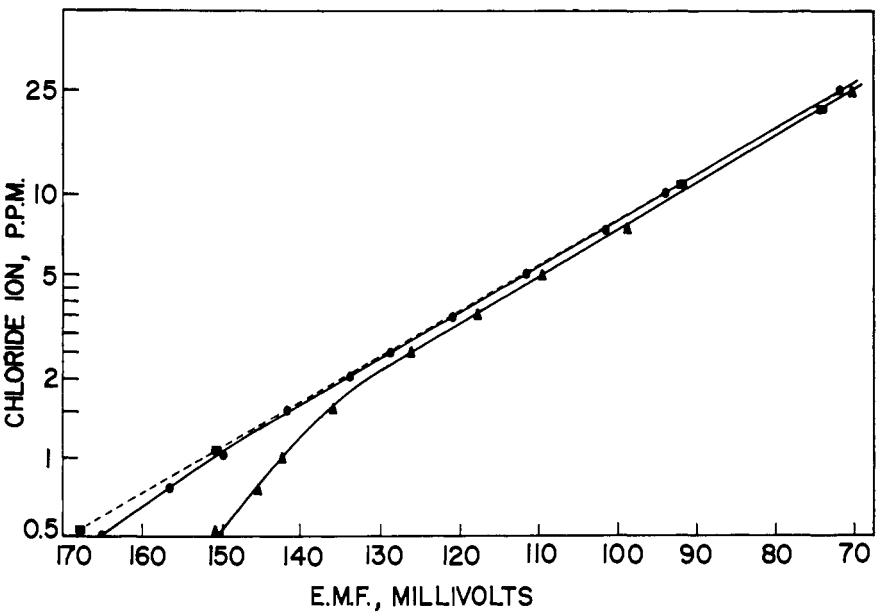

Figure 2. Effect of solvent systems on standard chloride curve

(▲–▲–▲, aqueous system; ●–●–●, 50% methyl Cellosolve system; ■–■–■, theoretical curve)

is obtained, warming gently if necessary. Stopper the flask, shake for 30 seconds and allow the phases to separate. Transfer the two phases into a graduated oil tube and completely remove the upper phase, using a small glass tube and suction. Save the lower aqueous phase for the analysis of chloride ion.

Chloride Ion Determination. Rinse the concentration cell with water and then with acetone. Dry the cell by drawing air through the center arm. Into one side arm add 0.2 ml. water; to the other side arm add 0.2 ml. of the salt solution. Into both side arms pipet 3.0 ml. of the sample. Rinse the two electrodes with water, shake off excess, and introduce the moist electrodes into the solutions. Fasten a hose to a needle valve fitted to a cylinder of nitrogen and adjust the flow to deliver a small stream of gas. Connect this hose to the center arm of the cell and mix the solutions with the stream of nitrogen. Disconnect the hose, and shut off the nitrogen tank. Place the cell in the water bath, making sure that the solutions in each arm are at the same level. Set the timer for 5 minutes. After $3^1/_2$ minutes, again introduce nitrogen into the center arm for one full minute, taking care to maintain a positive nitrogen pressure during the attachment of the hose. Then disconnect the hose and attach a small rubber bulb or rubber policeman to the center arm. Carefully draw both solutions into the center arm until a liquid junction is made, and immediately measure the e.m.f. to the nearest 0.1 mv. Obtain the p.p.m. of chloride from a standard curve prepared as follows. To convert p.p.m. chloride to an insecticide, such as DDT, multiply this value by 2 to obtain the p.p.m. DDT equivalent and read the p.p.m. DDT in the fat from the correction curve. For toxaphene the factor would be 1.5, and a correction curve for toxaphene would be needed.

Standard Curve. Weight 1.6482 grams of sodium chloride into a 1-liter volumetric flask and make to volume with water (stock solution A). Pipet a 50-ml. aliquot of this stock solution into another 1-liter flask and make to volume (solution B). Prepare a series of standards by pipetting 1, 1.5, 2, 3, 4, 5, 7, 10, 15, 20, and 50 ml. of solution B into 100-ml. volumetric flasks. This series of standards represents 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.5, 5.0, 7.5, 10, and 25 p.p.m. chloride in 10 grams of fat. To each flask, add 50 ml. of methyl Cellosolve and 5 ml. of concentrated nitric acid. Make to volume and determine the e.m.f. of these solutions under the same conditions. Plot e.m.f. values of the standards versus p.p.m. chloride ion (Figure 2). Since the per cent chlorine in DDT is approximately 50%, DDT concentration can be obtained by multiplying the chloride value by 2.

Correction Curve for DDT. Prepare fat samples with known amounts of DDT. Determine the chloride content using the standard curve. Correct for the blank, calculate the DDT equivalent and plot DDT added versus DDT found (Figure 3).

Results and Discussion

Standard Curve. The standard curve prepared with pure sodium chloride deviates from a straight line at the lower concentrations (Figure 2). The standard curve is reproducible not only with the same set of electrodes, but with other electrodes prepared under similar conditions. Because of the logarithmic nature of the standard curve, the region from 1 to 10 p.p.m. chloride is more sensitive (larger e.m.f. change per p.p.m.) than the 10 to 20 p.p.m. region. The blank must be kept as low as possible so that small changes in chloride will give an e.m.f. reading large enough to be significant. Reagents that contained chloride or organically bound halogen, particularly certain brands of xylene and liquid ammonia, in some cases gave blanks of 10 to 15 p.p.m. chloride.

The standard curve is influenced by the chloride ions contributed by the dissolution of silver chloride electrode. At 25° C., dissolution of silver chloride contributes 0.44 p.p.m. chloride. This amount of contamination caused the standard curve prepared in water solutions to deviate from a straight line at concentrations below 2.5 p.p.m. (Figure 2). Suppression of the ionization of silver chloride to some degree is possible if methyl Cellosolve is used in the solvent system (*2*). A 50% methyl Cellosolve system decreases the slope of the curve in the 0.5 to 5 p.p.m. region, thereby increasing the sensitivity.

The theoretical standard curve in Figure 2 was calculated by the formula

$$E = 0.059 \log \left[\frac{x + a}{x}\right]$$

where x is the unknown chloride ion concentration and a is the concentration of added chloride in 3.20 ml., the volume of solution in one of the side arms of the cell. A concentration of 350 p.p.m. in the final solution was found to be optimum; higher concentrations did not increase the slope, but did shift the e.m.f. to a slightly higher value (*6*).

Table I. Typical Recovery Data for DDT and Chloride Added to Lard[a]

Description	E.M.F., mv.	Chloride, P.P.M. Measured	Chloride, P.P.M. Recovered	DDT,[b] P.P.M. Measured	DDT,[b] P.P.M. Corr.[d]	E.M.F., mv.	Chloride, P.P.M. Measured	Chloride, P.P.M. Recovered	DDT,[c] P.P.M. Measured	DDT,[c] P.P.M. Corr.[d]
Reagents	145.2	1.2	...	...	...	113.5	4.8	...	...	...
	144.0	1.3	...	...	...	...	...	...	...	...
Inorganic chloride	131.5	2.2	...	...	...	...	...	..	...	...
	132.5	2.2	...	...	...	...	...	...	...	...
Lard	110.0	5.4	0	0	0	102.9	7.1	0	0	0
	109.8	5.4	...	...	...	103.4	7.1	...	...	...
Lard + 10 p.p.m. DDT	92.1	11.0	5.7	11.4	9.1	87.4	13.5	5.9	11.8	9.4
	92.0	11.1	...	...	...	88.6	12.5	...	...	...
Lard + 5 p.p.m. Cl^-	91.2	11.5	6.2	12.4	10.0	...	...	...	...	...
	90.8	11.6	...	...	...	...	...	...	...	...

[a] Low in pesticide residue. [b] Samples were not subjected to heat rendering. [c] Samples were heat rendered. [d] Corrected using calibration curve (Figure 3).

The presence of nitric acid not only minimizes emulsion formation during the removal of the chloride ion from the reduced fat, but makes the reading sharper for the 50% methyl Cellosolve solvent system. No effect on e.m.f. was noted when the nitric acid concentration was increased to 10%.

Recovery of DDT in the Presence of Lard. Recovery of DDT was based on comparison of chloride values obtained after reduction of DDT in lard, with values obtained from lard fortified with chloride ion in amounts equivalent to those expected from complete reduction. On this basis, over 90% of the added DDT was reduced to the chloride ion (Table I). Recovery studies of DDT added to lard samples prior to the rendering step compared quite well with the previous results. Despite incomplete reduction, DDT recoveries at low concentrations are high. Studies with a pure chloride standard 2.5 p.p.m. Cl^- have shown that hexane causes the e.m.f. to shift, giving an apparent 0.5 p.p.m. chloride increase. Saponification of fat with sodium hydroxide in amounts equivalent to the added sodium increases the value by another 0.5 p.p.m. chloride. Consequently, the 2.5 p.p.m. Cl^- standard chloride curve gives an apparent value of 3.5 p.p.m. chloride; thereby accounting for the high values found for both added chloride and DDT. For maximum accuracy, a standard correction curve (Figure 3) is recommended.

Removal of Water-Soluble Chloride. Adipose tissue contains some water-soluble inorganic chloride which must be removed before the organic halide is reduced. This is simply accomplished by the rendering-filtration procedure described. Presumably, the rapid loss of water at this high temperature and the presence of the lipid-cellulose barrier prevents the aqueous chloride solution from coming through the filter paper and contaminating the sample. This filter paper method can also be applied to rendered fats (Table II). The water-soluble chloride level of rendered samples, determined by potentiometric measurement of aqueous extracts, can be reduced to less than 0.5 p.p.m. by this procedure. No appreciable loss of DDT was found for samples carried through the rendering step. Other pesticides were not tried.

Silver–Silver Chloride Electrodes. No appreciable poisoning of the electrodes by trace amounts of lipides in the aqueous phase was noted. Rinsing the electrodes with acetone, followed by soaking for 10 minutes in water, did not change the e.m.f. of blanks or standard solutions.

E.m.f. measurements were stable for several minutes after the junction was made, provided that the solutions in the side arms were at the same level so that siphoning from one arm to the other did not occur.

Sodium Reduction. Anhydrous liquid ammonia can be as safely handled in the laboratory as any other volatile solvent. Addition of cold liquid ammonia to an ether solution of fat results in the quick freezing of the mixture, producing large particles or a completely solidified mass which trapped the sodium and prevented its reaction with the organic chloride. Preliminary slow cooling in a liquid ammonia bath (−33° C.), or dry ice and acetone, is preferred, since a finer precipitate is produced.

Table II. Effect of Filter Paper Rendering on Water-Soluble Chloride[a]

Sample	Water-Soluble Chloride: Before rendering, p.p.m.	Water-Soluble Chloride: After rendering, p.p.m.
Beef trimming	...	<0.5
Lard	2.2	<0.5
Commercial fat	81	<0.5

[a] Samples allowed to render through filter paper at a temperature of 120° C.

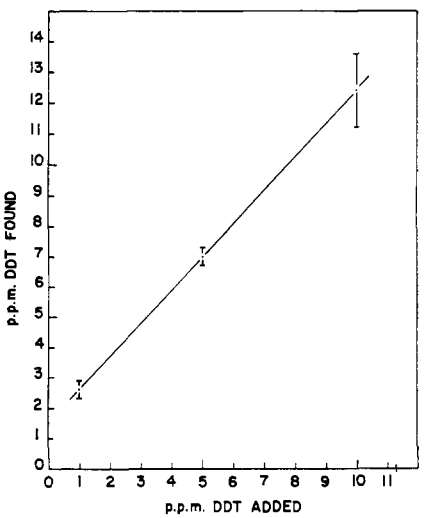

Figure 3. Correction curve for DDT added to lard prior to heat-rendering

(Standard deviation is based on four determinations)

Literature Cited

(1) Beckman, H. F., Ibert, E. R., Adams, B. B., Skovlin, D. O., J. AGR. FOOD CHEM. **6,** 104 (1958).
(2) Bertolacini, R. J., Barney, J. E., *Anal. Chem.* **30,** 202 (1958).
(3) Chablay, E., *Ann. chim. Paris* **1,** 469 (1914).
(4) Furman, N. H., Low, G. W., Jr., *J. Am. Chem. Soc.* **57,** 1585 (1935).
(5) Koblitsky, L., Adams, H. R., Schechter, M. S., J. AGR. FOOD CHEM. **10,** 2 (1962).
(6) Krzeminski, L. F., Landmann, W. A., Proceedings of the Residues in Foods Conference, Michigan State University, p. 55, 1961.
(7) Schmitt, R. A., Zweig, G., Division of Agricultural and Food Chemistry, 140th Meeting, ACS, Chicago, Ill., September 1961.
(8) Zweig, G., Sitlani, R. N., Division of Agricultural and Food Chemistry, 140th Meeting, ACS, Chicago, Ill., September 1961.

Received for review January 22, 1962. Accepted May 23, 1962. Journal Series Number 226, American Meat Institute Foundation.