A Screening Method for the Determination of Organically Bound Chlorine from Certain Insecticides in Fat

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This method is designed primarily for screening residues of DDT and the more common chlorine-containing pesticides in amounts above 5 p.p.m. in fat. The rendered fat sample dissolved in hexane is cleaned up by shaking with strong sulfuric acid, then with a potassium bicarbonate–potassium permanganate solution. After concentration, the solution is treated with dispersed sodium and the resulting inorganic chloride is determined by an automatic coulometric titration.

OVER 36 MILLION cattle and sheep and over 70 million hogs are slaughtered in the United States each year for human consumption under the jurisdiction of the Meat Inspection Service, U. S. Department of Agriculture. To maintain the high quality of meat products, it is desirable to have simple, rapid screening methods to detect the presence of chlorine-containing insecticides in fatty tissues where they tend to accumulate. Tolerances have been set by the Food and Drug Administration at 7 p.p.m. for DDT, lindane, and toxaphene and at 3 p.p.m. for methoxychlor in beef fat. Any insecticide for which a tolerance has not been set is considered to have a zero tolerance. Specific and sensitive procedures for many of the insecticides are available, but they are intricate and time-consuming.

The present method based on the determination of total organically bound halide, although nonspecific, was designed for screening several common insecticides at levels above 5 p.p.m. The apparatus and technique are simple and the method can be adapted to the analysis of large numbers of samples. Those samples which show excessively high levels of chloride can then be examined in detail by the more sensitive and specific analytical and paper chromatographic procedures.

In this method, rendered fat dissolved in hexane is shaken gently with a mixture of concentrated and fuming sulfuric acids (*10*). The hexane solution is then washed with a potassium bicarbonate–potassium permanganate solution, concentrated, and treated with dispersed sodium (*4*). The resulting inorganic chloride is determined by an automatic coulometric titration (*1*). The sensitivity of the titrator employed has been extended from the 9 μg. (0.25 μeq.) of chloride claimed by the manufacturers down to 1 μg. by the use of 35% acetic acid instead of water.

Since this work was completed, screening methods for chlorinated insecticides in fat have been presented by Krzeminski and Landmann (*2*) and Schmitt and Zweig (*11*), and for toxaphene in milk by Zweig and Sitlani (*14*).

Experimental

Reagents. Hexane. Any commercial brand with a low chloride blank; Esso hexane has been found satisfactory. If the blank is high, the hexane should be refluxed with some dispersed sodium and a very small amount of isopropyl alcohol, and distilled, with the first and last portions discarded.

Acid mixture. Three parts by volume of concentrated sulfuric acid and 1 part of 30% fuming sulfuric acid, containing 10 grams of sodium sulfate per 100 ml. of mixture.

Dispersed sodium, 25%. Dilute 50% sodium dispersed in mineral oil (Acton Laboratories, Newark, N. J.; Gray Chemical Co., Inc., Gloucester, Mass.; or equivalent) with an equal weight of white, light, paraffin oil (Fisher Scientific Co., Pittsburgh; or equivalent).

Nitric acid–acetic acid mixture. Add 65 ml. of concentrated nitric acid to 1000 ml. of glacial acetic acid and mix thoroughly.

Gelatin reagent. Gelatin–thymol blue–thymol—60:1:1 (usually supplied with the automatic chloride titrator). Dissolve 6.2 grams of reagent in 1000 ml. of hot water. Transfer to small vials in portions sufficient for 1 day's analyses. Keep under refrigeration. Liquefy each day's portion by immersing the vial in warm water.

Standard solutions. Solutions of sodium chloride in 35% acetic acid containing 12.5, 25.0, 50.0, and 100.0 μg. of chloride (Cl^-) per 4.00 ml. Also standard solutions in hexane of various chlorinated hydrocarbon insecticides to check recoveries.

Apparatus. Titrator. Automatic chloride titrator (Cotlove) (*1*) and special titration vials (American Instrument Co., Silver Spring, Md.; Buchler Instruments, Inc., New York, N. Y.; or equivalent).

Standard Laboratory Glassware. Clean in the usual manner, rinse thoroughly with distilled water, and dry. Care should be taken not to touch the rims, lips, or inside of any glassware after cleaning in order to avoid contamination by chloride from the skin. Sodium bisulfite or metabisulfite solutions can be used to remove manganese dioxide stains.

Procedure. Preparation of Standard Curve. Transfer 4.00 ml. of 35% acetic acid solution (v./v. aqueous) and 4.00 ml. of each of the standard sodium chloride solutions to a series of the special titration vials. To each add 0.40 ml. of the nitric acid–acetic acid mixture from a buret and four drops of the gelatin solution from an eye dropper. Clean the electrodes with a fine scouring powder (Bon-Ami or equivalent) or silver polish and rinse thoroughly. Determine the number of seconds needed for the completion of the automatic titration of each sample with the relay set for a 10-μa. excess over the starting equilibrium value and with the titration-range regulator at

the low position. (Consult manual for the automatic chloride titrator used.) Plot micrograms of chloride *vs.* time in seconds on cross-section paper.

Preparation of Sample. Render approximately 80 grams of macerated beef fat in an oven set at about 130° C. until the water has been driven off (the melted fat will become clear), and filter the sample in the oven. Do not heat longer than necessary.

Extraction and Cleanup. Pour about 20 grams of melted fat into a tared, standard-taper, 500-ml. Erlenmeyer flask and weigh. Record the actual weight of sample taken. To the warm fat in the Erlenmeyer flask (rewarm slightly on the steam bath, if necessary) add 300 ml. of hexane with a cylinder calibrated to deliver, or a 300-ml. pipet. It is preferable not to permit any of the fat sample to come in contact with the ground portion of the flask; if it does, the hexane may be used to rinse it down. Swirl to dissolve the fat without permitting the solution to touch the ground-glass joint. Add 100 ml. of the sulfuric acid mixture, so that the ground-glass joint becomes wet, stopper the flask, mix the contents by inverting the flask completely and quickly six to ten times (refrain from vigorous shaking or violent agitation as this will increase the amount of emulsification, yet the inversions should not be so slow that the layers slip by each other without adequate mixing), and let stand for 30 minutes. Decant most of the hexane layer into a standard-taper 500-ml. Erlenmeyer flask, being careful not to transfer any acid or acid-hexane emulsion. Add 5 ml. of 20% w./v. potassium bicarbonate solution and 3 ml. of a saturated potassium permanganate solution and shake vigorously. Sufficient potassium permanganate solution should be used so that the lower layer remains purple after shaking. Let stand about 20 to 30 minutes until the hexane layer becomes clear. Occasionally, with some fat samples a faint turbidity may still be noticed in the hexane layer, but this may be ignored. With the aid of a graduated cylinder calibrated to deliver, or a pipet, transfer 150 ml. of the washed hexane solution to a 300-ml. Erlenmeyer flask. (A larger aliquot such as 200 ml. may be used if desired.)* By means of tweezers, add a glass bead, concentrate the solution on a steam bath to approximately 50 ml., and remove from the steam bath.* (*Asterisks represent places where the analysis may be interrupted until the following day, if desired.)

Dehalogenation of Extracted Insecticide and Determination of Chloride. Shake or stir the 25% dispersed sodium in oil and by means of a 2-ml. automatic pipet (Standard Scientific Supply Co., New York, N. Y.) add 2.0 ml. of the dispersion slowly to the warm, concentrated hexane extract and swirl the flask. Add 0.25 ml. of 99% isopropyl alcohol, swirl the flask at once and then occasionally over a period of at least 10 minutes. Add an additional 0.25 ml. of isopropyl alcohol, swirl the contents, place on the steam bath, and boil gently for at least 10 minutes. Remove from the steam bath, let cool, and cautiously destroy the excess sodium by adding 5 ml. of 99.9% methanol dropwise at first and then 1 ml. at a time, swirling the flask continuously. Add 2 ml. of water, eight drops of 30% hydrogen peroxide, mix, and replace the flask on the steam bath. Evaporate until the residue is viscous, rinse down the wall of the flask with a little hexane from a polyethylene wash bottle, and evaporate again. The heating periods should be long enough (at least 15 minutes each) to decompose the peroxide completely; otherwise the initial equilibrium reading on the automatic chloride titrator will be found to be higher than the usual 2 or 3 μa.* Cool, add 3 ml. of 35% acetic acid solution, and rinse down the wall of the flask with a few milliliters of hexane. Add slowly 1.0 ml. of concentrated nitric acid from a buret and swirl. Add two drops of 0.1% methyl orange indicator aqueous solution and continue the titration to a persistent pink color. Rinse down the wall of the flask with approximately 10 ml. of hexane and heat gently on the steam bath for a few seconds. Transfer the warm solution by means of a funnel into a standard-taper, 50-ml. graduated cylinder (recalibrated to contain 20.0 ml.) using 10 ml. of additional hexane and at least three 3-ml. portions of 35% acetic acid. Allow to cool, make the lower layer up to 20.0 ml. with 35% acetic acid solution, shake, and let the layers separate completely. (If the solution is allowed to stand overnight, the indicator color may change from pink to yellow due to oxidation by nitric acid, but this is of no consequence.) Aspirate off the upper layer and, by means of a pipet, transfer 4.00 ml. of the remaining lower layer to a special titration vial for the chloride titration. Any slight turbidity that may be present in the lower layer does not affect the results. Continue as in Preparation of Standard Curve beginning with "add 0.40 ml. of the nitric-acetic acid mixture." From the number of seconds required for the titration, determine the micrograms of chloride in the aliquot from the standard curve, and calculate as shown below.

A blank analysis should be run with all of the reagents to make sure that they are pure enough for this method. With the amounts of solvents, reagents, and aliquots indicated, such blanks do not show more than 50 μg. of chloride, which is equivalent to 2.5 p.p.m. of chloride based on a 20-gram fat sample. Check analyses should be run on a series of uncontaminated fat samples to obtain an average check, and recovery analyses should be run on uncontaminated fat samples to which known amounts of the insecticide to be determined are added.

Table I. Recovery of Some Chlorinated Insecticides Added to 20 Grams of Fat

Added, P.P.M.	Mean Found,[a] P.P.M.	No. of Detn.	Standard Deviation, P.P.M.	Mean Recovery, %
		DDT		
6.0	5.9	8[b]	0.3	99
10.0	9.8	7[c]	1.0	98
20.0	19.0	8[c]	1.0	95
		Toxaphene		
9.6	9.1	4[b]	0.4	94
10.0	9.5	8[c]	1.3	95
20.0	19.8	8[c]	1.2	99
		Lindane		
9.5	9.6	3[b]	0.7	102
10.0	8.6	7[c]	1.0	86
20.0	17.5	8[c]	0.9	87

[a] Corrected for fat and reagent analyses. [b] Pesticide Chemicals Research Branch Laboratory, Moorestown, N. J. [c] Pesticide Chemicals Research Laboratory, Beltsville, Md.

Calculation of Results

$$\text{Chloride, p.p.m.} = \mu\text{g. chloride in aliquot titrated} \times \frac{20}{4} \times \frac{300}{150} \times \frac{1}{\text{grams of fat sample}}$$

This equation is applicable only if the indicated aliquots are taken. The analytical results should be corrected by subtracting the average check value for uncontaminated fat. The values of chloride in parts per million may be converted to parts per million of any insecticide by multiplication by the appropriate factor (2.0 for DDT).

Recovery Data

Known amounts of insecticides dissolved in 200 ml. of hexane added to 20-gram fat samples were run by this procedure, and the results corrected for check fat analyses are shown (Table I). For DDT and toxaphene, the mean recoveries ranged between 94 and 99% for 6 to 20 p.p.m. of added insecticide. For lindane, the recoveries were somewhat lower, 86 to 102%.

Check analyses (in Moorestown, N. J., laboratory) on fat plus reagents gave an average of 4.3 p.p.m. of chlorine (10 determinations) on one sample of check fat and a blank of 2.1 p.p.m. (14 determinations) on reagents alone, calculated on the basis of a 20-gram sample of fat being used. The Beltsville, Md., laboratory, with a different fat sample and reagents, found 5.2 p.p.m. (11 determinations) and 1.7 p.p.m. (5 determinations), respectively.

Table II. Effect of Washing 30-Gram Samples of Clear, Rendered Beef Fat to Remove Inorganic Chloride

Sample Was Washed[a]	Cl Determined by Method,[b] P.P.M.
No Sodium Chloride Added	
No washing	1.27; 1.02; 1.33; 0.99
Prior to acid cleanup	0.93; 0.95
After $KHCO_3$ and $KMnO_4$ wash	1.23; 1.01
Sodium Chloride Added to Give 2360 P.P.M. of Chloride	
No washing	Over 5 p.p.m.
Prior to acid cleanup	0.86; 0.93
After $KHCO_3$ and $KMnO_4$ wash	Over 5 p.p.m.

[a] Four washes with 50-ml. portions of 0.01*N* silver nitrate followed by four washes with 50-ml. portions of distilled water.
[b] Corrected for reagent blanks.

Table III. Effect of Sulfuric Acid Treatment on Certain Insecticides in Absence of Beef Fat

Insecticide Used, 400 μg.[a]	Chlorine Theoretical,[b] %	Insecticide Found (A),[c] μg.	Insecticide Found (B),[d] μg.	Recovery $\frac{(B)}{(A)} \times 100$, %
Methoxychlor	30.8	425	359	85
Dieldrin	55.8	357	272	76
Heptachlor	66.4	414	458	110
Heptachlor epoxide	63.7	392	434	111
Chlordane	69.2	394	436	111
2,4-D ethyl ester	28.5	371	253	68

[a] Samples were technical grade, except for methoxychlor and heptachlor epoxide which were recrystallized samples.
[b] Per cent chlorine is given for the pure, active ingredient and may vary from one technical sample to another.
[c] By sodium dispersion procedure alone on 400 μg. of insecticide in hexane, without acid treatment or bicarbonate wash; average of two or three determinations corrected for reagent blanks.
[d] Complete procedure including acid treatment and bicarbonate wash; average of two or three determinations corrected for reagent blanks.

Another laboratory (*3*) analyzed a series of fat samples by the present procedure and also by a paper chromatographic method for chlorinated insecticides (*6*, *7*). A series of 11 fat samples which showed negative results by paper chromatography gave an average of 4 p.p.m. of chloride (fat plus reagents), whereas a series of 12 samples which showed a trace (less than 1 p.p.m.) of chlorinated insecticides by paper chromatography gave an average of 5 p.p.m. Various lots of reagents yielded blank analyses of 1 to 3 p.p.m. of chloride calculated on the basis of a 20-gram fat sample.

Check fat samples indicate a background net level (corrected for reagent blank) of 2 to 3 p.p.m. of halide by this procedure. To investigate this problem further, experiments were run on 30-gram check samples to which very large amounts (2360 p.p.m.) of chloride were added. The chloride, as sodium chloride, was added to the flask in water solution, the solution was evaporated to dryness on the steam bath with the aid of a gentle stream of air, and 30 grams of rendered, molten fat was added followed by hexane. The hexane solution was shaken with four 50-ml. portions of 0.01*N* silver nitrate solution followed by four 50-ml. portions of water. The aqueous layers were removed by aspiration. The effect of the same wash solutions when employed after the potassium bicarbonate–potassium permanganate wash was also tested; the separation of the layers was then accomplished in separatory funnels. The results are given in Table II. Apparently, clear rendered fat does not contain more than a few tenths of 1 p.p.m. of inorganic chloride and this trace can be removed by washing a hexane solution of the fat with dilute silver nitrate solution. Very large amounts of added chloride can interfere, but as much as 2360 p.p.m. of added inorganic chloride can be removed by washing with dilute silver nitrate solution. When it appears necessary to remove the very slight interference from the traces of inorganic chloride in clear rendered fat, the insertion of a washing step of the hexane solution with dilute silver nitrate solution may be included prior to the acid cleanup.

Tests on Other Pesticides. Methoxychlor, 2,4-D ethyl ester, and several other pesticides are soluble in or are attacked by strong sulfuric acid to different degrees, and could give low recoveries. To check the extent to which these compounds might be attacked, 400-μg. amounts of several common pesticides dissolved in hexane were put through the procedure without the addition of fat and compared with direct analyses of the same amounts of pesticides in hexane without the acid treatment or bicarbonate wash. The results are given in Table III.

Test of Rendering Procedure. When a fat sample is rendered in an oven at 130° C., some loss of the more volatile insecticides such as lindane could occur. To find whether a loss did occur, a mixture of 1 ml. of water and 20 grams of fat to which 151 μg. (7.6 p.p.m.) of lindane had been added was heated in an oven at 130° C. for 5 hours. The average recovery of lindane was 139 μg. (6.9 p.p.m.) or 92% (five determinations) as compared with 143 μg. (7.2 p.p.m.) or 95% (three determinations) when the mixture was not heated, but just allowed to stand at room temperature for 5 hours. The difference is not significant. In later work it was decided that filtration of the fat during the rendering step in the oven or immediately afterwards might be advantageous.

Discussion

The objective of this work was to develop a screening procedure for the determination of residues of the commonly used chlorinated pesticides in animal fat by a procedure that involved simple apparatus and technique. An analysis for total organically bound halogen seemed to be appropriate, but extremely high sensitivity was neither necessary nor advisable in a screening procedure.

Several approaches were tried. The use of a Wickbold quartz burner (*13*) to burn a solution of the fat in a solvent proved to be too slow. Maceration of the fat with acetone, acetonitrile, or dimethylformamide left fatty residues in the solvent which required additional cleanup. Solution of the fat in a mixture of concentrated and fuming sulfuric acids and extraction of the insecticide with a solvent resulted in excessive emulsification.

A simple, rapid cleanup could be obtained when rendered fat was dissolved in a solvent and when the fat was extracted by gentle agitation with a mixture of concentrated and fuming sulfuric acids as adapted from the work of Schechter, Pogorelskin, and Haller (*10*) and Sargent and Wood (*9*). With this procedure, the fat sample has to be rendered first, but no appreciable loss of lindane (one of the insecticides most likely to volatilize) could be detected after heating a sample of fat containing this insecticide for 5 hours at 130° C. Solvents must be chlorine-free and have low boiling points, such as hexane and benzene. Benzene was not used (although a better solvent than hexane) because it forms emulsions difficult to break and is harder to evaporate. Best results were obtained with a mixture of three parts of concentrated sulfuric acid and one part of 30% fuming sulfuric acid for the extraction. The addition of 10 grams of sodium sulfate to 100 ml. of the acid mixture reduced emulsion formation. Acid mixtures with a greater fuming sulfuric acid content sulfonated some of the insecticides.

The hexane and acid layers must be mixed thoroughly enough to ensure

virtually complete extraction without excessive emulsification. The degree of emulsification may vary from one fat sample to another. Either too violent or too gentle agitation must be avoided. With practice, the mixing can be made thorough enough without emulsion formation so that an aliquot of 150 to 200 ml. can be taken from the decanted hexane without difficulty.

The hexane solution is washed with potassium bicarbonate solution to neutralize any traces of acid carried over in the solvent and to remove hydrogen chloride formed during the acid treatment, if traces of inorganic chloride are present in the fat. Potassium permanganate is added to the bicarbonate solution to remove sulfur dioxide which is produced by the reaction of sulfuric acid with fat. The odor of sulfur dioxide is readily noticeable in the hexane before it is washed.

The dispersed sodium procedure for the dehalogenation of the insecticide was adapted from the work of Menville and Parker (*4*). This method decomposes organic, halogen-containing compounds in minutes rather than hours, as in the usual sodium–isopropyl alcohol (*8*, *12*) or sodium–*sec*-butyl alcohol (*12*) procedures. The dispersed sodium used was that recommended by Menville and Parker (*4*). It was diluted with mineral oil to facilitate its measurement and decrease drainage errors.

Hydrogen peroxide is used to oxidize any sulfide or sulfhydryl groups which would combine with silver ions to give erroneously high values in the chloride titration. Any excess hydrogen peroxide must be completely decomposed during the heating steps prior to the titration; otherwise high initial equilibrium points (higher than 4 or 5 μa.) are obtained which may interfere with proper operation of the titrator. The equilibrium reading at the start of the titration will usually be 2 or 3 μa.

Commercial automatic chloride titrators, of the type described by Cotlove, Trantham, and Bowman (*1*), are used for the titration. This instrument, designed for the determination of chloride in biological materials, automatically titrates chloride with silver ions by coulometric generation of silver ions and amperometric detection of the end point. These instruments are claimed to have a sensitivity as low as 9 μg. (0.25 μeq.) of chloride when the titration is conducted in solutions of 10% acetic acid, 0.1*N* with respect to nitric acid. Amounts below this are not detectable because the solubility of silver chloride is approached and also the detector electrodes do not reach equilibrium rapidly enough. The addition of ethyl or isopropyl alcohol or an increased concentration of acetic acid in the sample solution was found to overcome these difficulties. Sensitivity was increased to 2 μg. per 4 ml. or 1 μg. per 2 ml. by using 35% acetic acid instead of water.

It is now virtually impossible to find fat samples completely free from trace residues of insecticides, and the fat samples used in these studies did indeed show traces of insecticides by paper chromatographic studies (*5*). However, the magnitude of the check analyses indicated the presence of more than trace amounts of halogen-containing substances. At present, the amounts found in the check fat samples are not readily explainable, but may be due to some slight carry-over of colloidally suspended inorganic chloride in the rendered fat and in subsequent steps, to the presence of unknown, naturally occurring halogenated compounds in fat, or to some as yet other undetermined type of interference. Although the causes for the apparent halide content of some check fat samples of 2 to 3 p.p.m. are not known, the procedure can nevertheless be used to screen for the more common chlorine-containing insecticides at levels above 5 p.p.m.

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INSECTICIDE RESIDUES

Residual Studies in Connection with Successive Applications of Heptachlor for Imported Fire Ant Eradication

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The imported fire ant, *Solenopsis saevissima richteri* Forel, is estimated to infest more than 21,000,000 acres in nine southern states. In 1957, the Congress appropriated $2,400,000 to commence the eradication of this pest. In a program of this size, even slight reduction in cost per acre can result in large savings. The present paper is concerned with residue studies aimed at determination of the minimum practical dosage of insecticide that can be used to accomplish eradication.

Lofgren (*7*) has reported that heptachlor is one of the most toxic insecticides tested against the imported fire ant. Heptachlor and dieldrin, at the rate of 2 pounds per acre in granular form, were the insecticides recommended for eradication purposes at the start of this program. The recommendations were based on research work conducted by the states of Mississippi and Alabama and the U. S. Department of Agriculture.

Recent work (*2–5*, *8*) has pointed out that heptachlor is lost very rapidly following application to the soil. Lofgren and coworkers (*6*) showed that two consecutive applications, each of 1/4 pound of actual heptachlor, spaced 3 or 6 months apart, are successful in the eradication of fire ants from small plots. Since it is physically impossible to treat the entire imported fire ant–infested area in the southern states in a short time, it was decided to determine the insecticide residue in the soil following