

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 μ a.) 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 μ a.

Commercial automatic chloride titrators, of the type described by Cotlove, Trantham, and Bowman (7), 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 μ g. (0.25 μ eq.) 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 μ g. per 4 ml. or 1 μ g. 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 colloiddally 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

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

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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

Insecticide residue studies were undertaken in connection with the eradication of the imported fire ant, *Solenopsis saevissima richteri* Forel. These tests were part of an attempt to find ways to reduce the amount of insecticide applied with no resulting loss in the length of the effective residue. Two successive applications of small amounts of heptachlor gave as good fire ant control as one application of larger amounts. Chemical analysis of soil shows that the insecticide residue following two successive $\frac{1}{4}$ -pound treatments of heptachlor is equivalent after 10 months to the residue following a single 1-pound treatment. Multiple applications have the added advantage that the number of application skips is reduced.

Table I. Residues of Heptachlor and Heptachlor Epoxide Found in Soil Following Consecutive Applications

(First application Jan. 26-28, 1959. Plots all sampled Nov. 24-25, 1959.)

Amount Applied		Date of Second Application	Residues, P.P.M.			% Remaining
Pounds/acre ^a	P.p.m. ^b		Heptachlor	Heptachlor epoxide	Total	
2	6.0	None	0.26	0.60	0.86	14.3
1	2.94	None	0.04	0.24	0.28	9.5
$\frac{1}{2}$	1.25	None	0.04	0.14	0.18	14.4
$\frac{1}{2}$	3.1	4-16	0.02	0.26	0.28	9.0
$\frac{1}{2}$	2.62	8-8	0.14	0.26	0.40	15.2
$\frac{1}{4}$	0.78	None	0.03	0.06	0.09	11.5
$\frac{1}{4}$	1.50	4-16	0.06	0.20	0.26	17.3
$\frac{1}{4}$	1.29	8-8	0.07	0.20	0.27	20.9
$\frac{1}{8}$	0.42	None	0.03	0.03	0.06	14.3
$\frac{1}{8}$	0.84	4-16	0.02	0.06	0.08	9.5
$\frac{1}{8}$	0.75	8-8	0.03	0.09	0.12	16.0

^a Each treatment; nominal amount applied.

^b Calculated as follows:

$$\text{P.p.m.} = \frac{W \times A \times 1,000,000}{w} = 3WA$$

where

W = weight of formulation applied in pounds (both applications), per acre

A = percentage toxicant in formulation

w = dry weight of soil (sandy loam) in 1 inch = 330,000 lb. per acre.

such applications, in order to determine the approximate length of protection provided by this treatment.

Methods

The plots used for the study were from two series of experiments for imported fire ant control. In series I, two applications of $\frac{1}{8}$, $\frac{1}{4}$, or $\frac{1}{2}$ pound per acre of heptachlor were tested at treatment intervals of 3 or 6 months. The biological results of this test have been reported (6). In the second series, which was part of a test on the effect of time of application on imported fire ant control with heptachlor, dosages of $\frac{1}{4}$, $\frac{1}{2}$, 1, and 2 pounds per acre were tested. Three replicates were run in the first series and two in the second. Both series of tests were conducted in the same field, a bahia grass pasture, and were applied with a Gandy fertilizer distributor over a period of 3 days. The plots in series I were 2 acres in size and in series II, 1 acre. The modified sampling and analytical technique described in previous investigations (2, 8) was used. There were 44 plots in the

test, including five untreated ones used as blanks.

Materials

The heptachlor was applied in granular formulations. The concentrations were adjusted so that for the $\frac{1}{8}$ -, $\frac{1}{4}$ -, and $\frac{1}{2}$ -pound dosages in test series I, 10 pounds of total formulation per acre could be used, and for the $\frac{1}{4}$ -, $\frac{1}{2}$ -, 1-, and 2-pound dosages in series II, 20 pounds of total formulation. This was accomplished by providing 1.25, 2.5, and 5% heptachlor granules for series I, and 1.25, 2.5, 5, and 10% for series II. The material varied only in heptachlor content. The granules were 24- to 48-mesh Attapulugus clay, extruded and dried. The deactivator used was Deactivator H (technical diethylene glycol). The heptachlor, in quantity sufficient to treat the required weight of clay, was dissolved in 10% (by weight of finished product) heavy aromatic naphtha, a petroleum fraction consisting of methylated aromatics boiling from 320° to 660° F. This material met specifications current at the time (7). The mixture was heated and stirred to

facilitate solution and sprayed while still warm onto the clay, which was tumbling in a modified cement mixer. The deactivator was applied as a spray, either previous to or with the insecticide. These formulations were all commercially prepared, except for the 1.25% heptachlor. All were analyzed at time of application.

Results and Discussion

In Table I are given the results of residue analyses of the various treatments. The determinations for the identical treatments were averaged. None of the figures deviated sufficiently from the median to be open to question. The times of application, rates, and amounts of heptachlor and heptachlor epoxide found, with percentage remaining of that applied, are listed.

Previous studies (2-5, 8) showed that there was an initial, very rapid, loss of heptachlor followed by a much slower loss of that remaining, with simultaneous conversion of a part to heptachlor epoxide. The present study confirms these findings, in that there is little difference in residue, if the second application went on 3 or 6 months following the first.

The success with consecutive quarter-pound applications of heptachlor for fire ant eradication is now more readily understood, as the two treatments yield a residue almost equal to a single 1-pound treatment applied at the beginning of the study. The efficiency with which an effective insecticidal residue is maintained in the soil is the important feature in the eradication efforts against the imported fire ant. It is apparent from this study and, in light of the very rapid loss of heptachlor from the soil, that two light applications of heptachlor at 3- to 6-month intervals are more efficient than one heavy application.

Since the two successive $\frac{1}{4}$ -pound applications yield such a relatively high residue, the added advantage to be gained by two applications with consequent lower likelihood of skips makes this treatment the one of choice for imported fire ant eradication, confirming

the conclusions of Lofgren, Adler, and Barthel (6).

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INSECTICIDE RESIDUES IN MEAT AND MILK

Feeding of Malathion to Cattle: Residue Analyses of Milk and Tissue

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The use of malathion for insect control in stored cattle feed and forage requires knowledge of the possible excretion of the insecticide in milk, or of its accumulation in tissue, at higher levels of cattle feeding than have heretofore been investigated. No malathion was found in milk of cows fed up to 800 p.p.m. of the insecticide based upon a 12-pound daily ration of dairy chow. Hay was permitted *ad libitum*. Similarly, no malathion was detected in blood, liver, kidney, heart, muscle, or fat of ruminating calves fed 200 p.p.m. of the insecticide, based on total food intake, for 41 to 44 days. Some malathion was found in the liver of two calves that were sacrificed after 14 days at this level of intake. Statistical analysis permits the conclusion that, at a 95% level of confidence, 95% of such samples will contain less than 1 p.p.m. of malathion. No ready explanation of why malathion should be present after the shorter interval of feeding is apparent.

MALATHION, *S*-[1,2-bis(ethoxycarbonyl)ethyl]*O,O*-dimethyl phosphorodithioate, is a broad-spectrum insecticide that has widespread use in plant and animal health areas. Previous investigations to determine the occurrence of malathion residues in milk and meat of cattle have been concerned with either dermal application of the insecticide, or its feeding at relatively low levels in the diet. Smith *et al.* (7) found that two cows, fed malathion at the established tolerance level for alfalfa (8 p.p.m.) for three weeks, did not excrete the insecticide in milk, nor was it detectable in blood, brain, liver, kidney, round, or rib-eye of one animal that was slaughtered immediately upon termination of the experiment. Gjullin, Scudder, and Erwin (4) pastured cows on alfalfa sprayed with known amounts of malathion, and subsequently failed to detect any in milk. It must be presumed that the animals ingested some of the insecticide, but the amount is unknown. Wells *et al.* (8), using 0.5 and 1.0% malathion sprays and 4% dust for controlling flies in dairy barns and on dairy cattle, found that malathion is excreted in milk under these conditions in varying small amounts, the magnitude of which

depends upon the type of treatment, and the interval before sampling. However, Goulding and Terriere (5), using a 4% dust for horn fly control on dairy cattle, reported that only occasional milk samples collected 12 to 60 hours after treatment contain any detectable amount of malathion. The two results considered positive by these authors were 0.01 p.p.m. from which it would appear that they attribute an unusually high degree of precision to the analytical procedure. Some malathion (0.03 to 0.05 p.p.m.) was found in milk when a 10% dust or a 0.5% aqueous dispersion of a wettable powder was employed. The report of Claborn *et al.* (2) on the application of 0.5 and 1% malathion sprays to cattle shows residues of 0.08 to 0.36 p.p.m. in milk 5 hours after spraying. The duration of a detectable residue was 3 days. These authors were unable to find malathion (< 0.5 p.p.m.) in fat of cattle that had received 16 sprays at 1-week intervals, when the fat was sampled 1 week after the last spraying.

Interest in the use of malathion for control of insects in stored cattle feed—e.g., citrus pulp—prompted the present investigation. In such uses, insecticide residues on the feed may attain relatively high levels; therefore, it is necessary to determine the amount of malathion, if any, in milk and edible tissues of cattle under such conditions.

Experimental

Analytical Method. The colorimetric method of Norris *et al.* (6) was used for the determination of malathion in milk, muscle, fat, and liver. Kidney was assayed by the same procedure as described for liver, with the exception that the sodium sulfate-hydrochloric acid wash was omitted. Heart was treated by the procedure described for fat-free meat. Blood (heparinized) was assayed by extracting 100 ml. with two successive 200-ml. portions of carbon tetrachloride, combining the extracts, and proceeding from that point as with fat-free meat.

Pretreatment milk samples from each of the cows used in this study, approximately 6 gallons in all, were pooled in order to obtain samples for establishing a blank value and determining the efficiency of malathion recovery. The composite sample was separated into 500-gram portions; two 500-gram samples were required for one assay. Twenty samples were freeze-dried for blank determinations, while the remaining samples were so treated after the addition of known amounts of malathion. Results of the blank and recovery determination are presented in Table I.

In the case of blood and meat, blank and recovery values were determined using tissues obtained from ruminating calves of approximately the same ages and breeds as those used in the feeding

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