lower rate of methyl bromide usage. The tolerance of cabbage, cipollini, garlic, and yams to such fumigation has been discussed by Roth and Richardson (*9*, *10*).

Residues in yams (Table III) from atmospheric pressure fumigation (NAP) are variable, but generally lower at 55° F. than at 80° to 86° F. The residue from the vacuum fumigation at 80° F. is about the same as would be produced by an atmospheric fumigation at that temperature. Peas show little temperature dependence in this study. The residue levels in the honeydew melons are all below the level of sensitivity of the x-ray fluorescence method used.

Fumigation of apples results in a very low rate of bromide residue accumulation (Table III), similar to peaches and plums (Table I). The residue in blueberries, slightly higher, is comparable with grapes and cherries (Table I). Cabbage accumulates residue at a significantly higher rate, comparable with carrots and peppers (Table I) and yams (Table III). Although the results are not directly comparable because of different chamber loading, the residues on cabbage after 15-inch sustained vacuum fumigation appear slightly higher than those after atmospheric pressure fumigation in two paired tests at 34° and 51° F.

The results reported here are generally in good agreement with those of Dudley (*3*), who also fumigated carrots, apples, and sweet potatoes with methyl bromide at the rate of 2 pounds for 2 hours.

This series of fumigations included some preliminary tests of ethylene dibromide (EDB) alone on blueberries and in combination with methyl bromide on apples and blueberries. The efficiency of these fumigants is being studied separately. The results given here are included merely for comparison of the residues resulting from this type of schedule. The possibility of ethylene dibromide per se remaining in the samples at the time of analysis has not been studied.

The data indicate that, on a nearly equal weight basis, there is less bromide residue from ethylene dibromide than from methyl bromide. This is not unexpected because of the relatively greater reactivity of the latter.

It is concluded from this study that no excessive residues of bromide will result from fumigation of these fruits and vegetables with methyl bromide following quarantine schedules.

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HEPTACHLOR IN ALFALFA

Distribution, Movement, and Persistence of Heptachlor and Its Epoxide in Alfalfa Plants and Soil

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The development of more sensitive analytical procedures for the detection of insecticide residues necessitates the re-evaluation of application procedures, especially where food products are concerned which traditionally are assigned a zero tolerance. Electron-capture gas chromatography revealed the presence of residues of heptachlor epoxide and dieldrin in milk produced in areas where these insecticides were being used to control the alfalfa weevil. A statewide testing program conducted by the University of Maryland involving over 1500 analyses of dairy feeds indicated that alfalfa treated the previous fall was the only significant source of the residue in milk (*5*).

Lichtenstein and coworkers have reported the translocation of chlorinated hydrocarbons from soil to a variety of plants (*9*, *11*) and the epoxidation of heptachlor and aldrin by plant tissue (*6*, *10*). Insecticide in the leaves and stems of pea plants was shown to be the result of absorption through the roots and not of adsorption of vapor by the aerial parts of the plants (*10*). Carrots were reported not only to absorb more insecticide than some other plants, but to concentrate it in their roots (*6*, *7*). The retention of heptachlor by the soil was greater when alfalfa had been used as a cover crop (*8*, *11*). Terriere and Ingalsbe (*13*) reported the translocation of heptachlor, aldrin, and dieldrin in potatoes. Traces of dieldrin in oil and meal from cottonseed produced in treated soil were reported by Randolph and coworkers (*12*). Eden and Arthur (*3*) found small residues of DDT and heptachlor in soybeans grown in soil treated at the time of planting, but concluded that there had been no translocation. Hardee and coworkers (*4*) found detectable residues of dieldrin in alfalfa 32 months after treatment with 3 to 5 pounds per acre. The soil contained most of the residual insecticide in the top 1 inch and it was concluded that the mechanism of contamination was by splashing onto the plant. Contamination of alfalfa is also considered to be the result of dust created during haymaking operations (*2*).

The fall application of 1 pound per acre of heptachlor mixed with granular fertilizer resulted in residues in alfalfa. Second and third cuttings had greater residues than the first. Silage and green chop had greater residues than hay. Commercial dehydration reduced the residue by over 50%. Mechanical contamination by soil was negligible. Analyses of entire plants showed the crown to be most heavily contaminated, but even the roots had more residue than the top growth. Soil residue at various depths was much less than in the plant and was mostly in the top 1 inch. Disappearance from the soil 10 months after application was 60 to 80%. Greenhouse experiments and root dissection confirm the internal movement of the insecticide in the plant after absorption from the soil. A reduction in residue in alfalfa of about 80% can be anticipated in the second growing season after the last application.

The present investigation was directed toward the elucidation of the nature, movement, and persistence of heptachlor in alfalfa plants and associated soil which had received the recommended application in granular form the previous fall.

Experimental

Field Study. Two areas at the University of Maryland Agronomy-Dairy Forage Research Farm were used for this work. One was a two-year-old stand of alfalfa that was treated with 1 pound of heptachlor per acre in November 1962 and again in November 1963 (field 29). The second field (28) was newly seeded to alfalfa in the fall prior to sampling. In November, 1 pound of heptachlor was applied to the seedling stand. In all cases heptachlor was mixed with granular fertilizer, which was applied at a rate of 800 pounds per acre. Both fields were excellent dense stands of Williamsburg alfalfa.

The preharvest samples referred to in Table I were taken from the first year stand (field 28) when the plants were in the early bud stage. The direct cut sample was hand-clipped with shears and was not allowed to touch the soil. The plants were thoroughly washed before being analyzed. The second preharvest sample was also hand-cut with shears, but was rubbed on the contaminated soil to simulate the soil exposure conditions experienced in a normal hay raking and baling operation. The subsequent samples referred to in Table I were from the two-year-old stand and were obtained through the normal farm operation. Sample 9 was processed through a nearby commercial dehydrating plant.

The samples referred to in Table II A were from the second-year stand. These plants were dug intact and were in the bloom stage with approximately 18 inches of growth.

The samples referred to in Table II B were obtained from field 28 with approximately 12 to 15 inches of growth in early October. These were dug using the tractor-mounted soil core sampler described by Boehle and coworkers (*1*). By centering this core tube over an individual plant, the plant, encased in a 4-inch-diameter, undisturbed core of soil, was removed. The core removed was 12 inches deep.

Table I. Residues of Heptachlor and Heptachlor Epoxide in Alfalfa as Influenced by Harvest and Postharvest Treatment

No.	Sample Description	Residue, P.P.M. Heptachlor	Hept. epoxide	Total
1	Preharvest, direct field-cut, water-washed	0.02	0.16	0.18
2	Preharvest, soil contaminated	0.06	0.13	0.19
3	First cutting alfalfa hay	0.04	0.16	0.20
4	Second cutting alfalfa hay	0.06	0.17	0.23
5	Third cutting alfalfa hay	0.03	0.24	0.27
6	Fourth cutting alfalfa hay	0.01	0.08	0.09
7	First cutting, alfalfa silage[a]	0.03	0.24	0.27
8	Second cutting, green chop, dried at room temperature[b]	0.04	0.28	0.32
9	Second cutting, green chop, dehydrated[b]	0.02	0.12	0.14

[a] Residue based on dry weight.
[b] Second cuttings obtained from field other than used in previous samples.

Table II. Distribution of Heptachlor in Entire Alfalfa Plants and Soil

Sample Description	Residue, P.P.M. Heptachlor	Hept. epoxide	Total
A. Entire Alfalfa Plants			
Top 2- to 4-inches of plant	0.02	0.18	0.20
Middle of plant	0.02	0.30	0.32
Bottom of plant	0.02	0.36	0.38
Crown	0.18	1.60	1.78
Roots	0.01	0.40	0.41
B. Entire Alfalfa Plants and Associated Soil from Field Treated One Year Only			
Upper half, top growth	0.02	0.20	0.22
Lower half, top growth	0.02	0.34	0.36
Crowns	0.32	1.44	1.76
Roots, 1-inch section below crown	0.05	0.63	0.68
Roots, mid-section	<0.001	0.16	0.16
Roots, tips, about 1 inch	<0.001	0.07	0.07
Soil, 0- to 1-inch depth	0.42	0.13	0.55
Soil, 1- to 3-inch depth	0.05	0.07	0.12
Soil, 3- to 6-inch depth	0.01	0.01	0.02
Soil, 6- to 8-inch depth	<0.001	<0.001	<0.001
C. Entire Alfalfa Plants and Associated Soil from Field Treated in Two Successive Years			
Top growth, 6 to 8 inches	0.02	0.32	0.34
Crowns	0.14	1.52	1.66
Roots, 0- to 2-inch section below surface	0.02	1.20	1.22
Roots, 2- to 9-inch section below surface	<0.001	0.25	0.25
Roots, 9-inch section below surface	<0.001	0.07	0.07
Soil, 0- to 1-inch depth	0.31	0.39	0.70
Soil, 1- to 3-inch depth	0.04	0.16	0.20
Soil, 3- to 6-inch depth	0.004	0.026	0.03
Soil, 6- to 9-inch depth	<0.001	0.004	0.004

Plants taken in late season from field 29 (Table II C) were also obtained with the core sampler.

Dormant alfalfa roots were dug in November from field 29 for analysis of the dissected roots.

Greenhouse Study. Dormant alfalfa plants were obtained from field 29 in December using the core sampler previously described. Plants were also dug from a pasture which had not been treated with heptachlor. Four of these plants in cores of contaminated soil were placed in large clay pots. The contaminated soil was washed from the roots of four other plants, which were potted in uncontaminated soil from a wooded area. Four plants from the untreated pasture were potted in contaminated soil from field 29. The 12 pots were set up on a greenhouse bench in plastic trays, and subirrigated to avoid splashing soil on the plant stems and leaves during watering. Initially, when the plants were potted, all stubble and top growth were clipped off as close to the crown as possible. The first harvest was made after 4 weeks, when approximately 10 inches of growth had accumulated. The second harvest was made 5 weeks later, when similar growth had occurred. At this time, the entire plants were removed and the roots were also analyzed.

Analytical. All plant and soil samples, except alfalfa silage, were dried at room temperature for 1 week before analyzing. After cutting away top growth, the crowns and roots were thoroughly washed in tap water to remove soil. They were subdivided while still wet and after drying were ground in a laboratory model Wiley mill using a coarse screen. The machine was cleaned with acetone after each sample was ground. Soil samples representing about 12 cores were ground in a mortar after drying. Hay samples were obtained by boring 30 bales using the Penn State forage sampler. Dissection was performed on a 2-inch section of root directly under the crown. The center section was removed by a small cork borer in one sample and by peeling away the outer skin with a scalpel in another. Extreme care was taken to avoid mechanical contamination of inner and outer sections.

Pesticides were extracted from plant material by blending 25 grams with 150 ml. of acetone in a Waring Blendor for 5 minutes and the liquid was decanted into a 300-ml. sintered glass suction funnel. Another 150 ml. of acetone were added to the blender jar, and the contents were again blended for 5 minutes, after which the entire contents were transferred to the filter funnel. The combined acetone extracts were mixed with 1500 ml. of distilled water in a 2-liter separatory funnel. The residue in the filter funnel was dispersed in 200 ml. of petroleum ether (30° to 50° C. fraction), collected in the original filtration flask, and added to the acetone-water mixture, and the separatory funnel was shaken for 5 minutes. The lower phase was discarded and, after two washings with water, the petroleum ether extract was brought to a standard volume such that 15 to 20 ml. represented 10% of the total extract.

Soil was extracted by dispersing 25 grams in 150 ml. of acetone in a 250-ml. volumetric flask which was kept in a water bath at 50° C. for about 2 hours and frequently shaken. The contents were transferred to a filter funnel, the acetone was removed by suction, and the soil was returned to the volumetric flask, again dispersed in 150 ml. of acetone, and returned to the bath for 1 hour. After removal of the second acetone extract, the combined extracts were mixed with distilled water and the soil was finally extracted with petroleum ether used subsequently to extract the water-acetone mixture as described for plant material.

An aliquot representing 2.5 grams of soil or plant material in 15 to 20 ml. of petroleum ether extract was added to a 20 × 400 mm. chromatographic column packed with Florisil (preactivated at 650° C., reactivated at 130° C.) by tapping to a height of 4 inches, capped with $^3/_4$-inch anhydrous Na_2SO_4, and prewetted with 40 to 50 ml. of petroleum ether. Pesticide was eluted with 200 ml. of 6% (v./v.) ethyl ether in petroleum ether collected in a 250-ml. beaker and evaporated to near-dryness by drawing a current of air over the surface. The residue was transferred to a 10-ml. graduated mixing cylinder with four portions of hexane and brought to a final volume of 5 ml. Marked losses of pesticides were observed if eluents were allowed to evaporate to complete dryness and remain thus for more than 2 to 3 minutes.

All residue analyses were completed by electron-capture gas chromatography with an Aerograph Model 680 instrument. The column was packed with Dow-11 on Chromosorb W, operated at 185° C. with a nitrogen flow rate of about 50 ml. per minute. Injections of standards and samples were in duplicate and standardized to 5 μl. The instrument was standardized before each use with a series of seven standards containing 0.02 to 0.5 nanogram per 5 μl. at a sensitivity such that 0.3 nanogram resulted in about 60% deflection of the recorder. The standard curve, based on peak height, was checked at least hourly while unknowns were run. All samples were brought within this operating range by appropriate dilution.

The procedures as described gave recoveries of more than 90% based on the addition of known standards.

Results and Discussion

Results of residue analyses of preharvest samples obtained with and without soil contamination are shown in Table I, 1 and 2. These results indicate that contact with soil during harvesting is not a major source of residue. The total residue is about the same for both samples. Prior to these findings it was considered that windborne or water-splashed soil particles of dust created during haymaking operations were the primary source of residue.

An interesting aspect was the continued presence of residue in all aftermath harvests of alfalfa. This is indicated by samples 3 to 6 in Table I. Similar results were observed among over 1300 samples of alfalfa analyzed for individual farmers, in which the second cuttings had about 20% greater residues than first cuttings.

Heat present during haymaking apparently results in some reduction of residue in alfalfa. This is shown by comparison of residues found in the hay, silage, and green chop samples (Table I). Similar results were observed among such samples analyzed for individual farmers. The effect of artificial drying in a commercial dehydrator is shown by comparison of samples 8 and 9 in Table I, indicating that over 50% of the residue can be removed by this process.

Samples of pearl millet and corn growing adjacent to alfalfa in a plot treated the previous fall with heptachlor at the rate of 1 pound per acre were analyzed at various stages of growth. Residues of heptachlor epoxide less than 0.06 p.p.m. were found in these plants, compared to more than 0.3 p.p.m. found in the alfalfa from the same plot. This suggested the possibility of pesticide storage in the alfalfa plant. The distribution of heptachlor and heptachlor epoxide in whole alfalfa plants is shown in Table II A. The top flowering portion of the plant contained somewhat less residue than the lower portion of the top growth, but the most marked difference was the large concentration found in the crown. The root also contained a greater residue than the top growth.

A more detailed study of the distribution of residue in the alfalfa plant and the soil associated with each root section is presented in Table II, B and C. The samples in Table II B were taken from a plot treated only once with heptachlor, whereas those in Table II C were from a plot treated in two successive years. The soil analyses show most of the residual pesticide in the top 1 inch with very little below a depth of 6 inches. The content of the pesticides was greater where the soil had been treated more than once, indicating some year to year accumulation.

Based on an application of 1 pound per acre, between 60 and 80% of the pesticide had disappeared from the soil within 10 months. The ratio of heptachlor to its epoxide was greater in the soil than in the plant. This suggests that the plant tissue, like animal tissue, is effective in converting heptachlor to its epoxide (*6, 10*). In all cases the concentration of residue was considerably greater in the plant than in the surrounding soil.

A root section that extended at least 9 inches below the surface was contaminated, but no pesticide was found in the soil at this depth (Table II C).

Table III. Residue in Dissected Root Section of Alfalfa Plants

Sample Description	Weight, Grams		Residue, P.P.M.		
				Hept. Epoxide	
	Wet	Dry	Heptachlor, dry	Dry	Wet
Bored centers	14	6.7	<0.001	0.43	0.20
Bored outer section	24	9.6	0.01	0.55	0.22
Peeled centers	30	13.4	<0.001	0.40	0.18
Peeled outer section	15	6.2	0.06	0.59	0.24

Table IV. Residue in Alfalfa Plants before and after Greenhouse Period

Sample Description	Residue, P.P.M.		
	Heptachlor	Hept. epoxide	Total
From treated field with soil intact			
First growth	0.01	0.06	0.07
Second growth	0.04	0.20	0.24
Roots, after second growth	0.03	2.50	2.53
From treated field in untreated soil			
First growth	0.01	0.04	0.05
Second growth	0.04	0.19	0.23
Roots, after second growth	0.02	0.89	0.91
From untreated field in treated soil			
Roots, before transplanting	<0.001	0.19	0.19
Roots, at end of greenhouse period	0.03	0.41	0.44

The accumulation was greater at all depths measured, in both the root and the soil, from the samples treated in two successive years and appears to be in the form of the epoxide, which is less volatile than heptachlor. The loss of insecticide from the soil is considered to be primarily by volatilization, the rate of which is probably influenced by soil type, weather conditions, and method of application. This is supported by the observation that the average residue of 150 alfalfa samples originating from fields receiving a fall spray application was 50% less than samples from fields given a fall granular application. In the former case much of the heptachlor probably left the soil shortly after time of application.

The residue present in alfalfa roots and crowns is not merely a surface contamination, but apparently a rather even distribution throughout the internal structure. This is demonstrated by the data presented in Table III, where inner and outer sections of roots prepared by two different methods were separately analyzed. Only the epoxide was detected in the inner sections. The penetration of the residue was further demonstrated by immersing crowns in acetone and then in petroleum ether for about 2 minutes. No difference in residue was observed in these crowns as compared to a similar lot washed with water only.

Two harvests were obtained from alfalfa transplanted from the field to the greenhouse (Table IV). The first harvest, taken about 4 weeks after transplanting, contained detectable residues, but the second growth harvested about 5 weeks later contained much greater residues. The residue in the roots grown in untreated soil decreased markedly during the greenhouse trial as compared to those in the treated soil. Soil analyses were not carried out in this experiment. Top growth from untreated plants potted in treated soil was too little for analysis, but analysis of the roots at the termination of the experiment showed a marked residue. These data demonstrate translocation of heptachlor epoxide in the alfalfa plant and the absorption from the soil to the root.

Accumulation of heptachlor or its epoxide in soil does not appear to present a long-term problem. Analyses of about 150 samples of alfalfa that had not been treated with heptachlor for the current growing season but had been for the previous one indicated an 80% reduction in plant residue.

Since this paper was submitted for publication, analyses of over 100 samples of alfalfa from the same individuals who submitted samples the previous year have verified the predicted reduction in insecticide residue resulting from the discontinued use of heptachlor. Similar results were obtained concerning the former use of dieldrin.

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