

Residues in Alfalfa and Soils Following Treatment with Technical Chlordane and High Purity Chlordane (HCS 3260) for Alfalfa Weevil Control

H. Wyman Dorough,* Raymond F. Skrentny, and Bobby C. Pass

Alfalfa was sprayed with technical chlordane and with a high purity chlordane (HCS 3260) which contained 98+ % α - and γ -chlordane. The active ingredients (AI) of technical chlordane consisted of 14% each of α - and γ -chlordane, 5% heptachlor, and 67% related compounds. Analyses were conducted for α -chlordane, γ -chlordane, heptachlor, and heptachlor epoxide which considered over 98% of the AI of HCS 3260 but only 33% of the AI of technical chlordane. Total ppm of these products

deposited on alfalfa treated with the technical chlordane and HCS 3260 at a rate of 1 lb AI/acre were 29 and 130, respectively. In all tests, 95% of the residues on the alfalfa had disappeared after 21 days. Three days of field-curing freshly cut alfalfa reduced the level of residues by about 55%. Maximum total residues were 0.6 ppm in soil of alfalfa treated with 2 lb AI/acre of HCS 3260. By 21 days these residues had declined by 50%, and by 180 days 83% of the residues had dissipated.

Technical chlordane is a complex mixture of polychlorinated bicyclohydrocarbons consisting of 60 to 75% isomers of chlordane and 25 to 40% of related compounds (Martin, 1968). Modern chromatographic techniques allow the separation of technical chlordane into a series of components which are extremely sensitive to electron capture detection. Using glc parameters described later in this paper, at least nine such products were resolved (Figure 1) from technical chlordane produced by Velsicol Chemical Corp. under the name Belt-72ECF.

Problems created by the presence of a variety of components in a biologically active material in regard to evaluation of its chemical and toxicological properties are apparent. In fact, this has been a subject of interest and periodic concern with chlordane since its introduction in 1945 (Ingle, 1965). A related problem, and one which has particular significance because it hinders our ability to monitor the environment and food products for pesticides residues, is the difficulty encountered in attempting to quantitate total chlordane residues by glc techniques.

While it has been possible for several years to obtain excellent recovery of chlordane from fortified samples, several workers have shown that weathered residues change in the number and size of gas chromatographic peaks (Klein and Link, 1967; Thurston, 1965). Estimates of the total level of residues on a weathered substrate were based on an average concentration calculated from several of the glc peaks. More recently, certain known components of chlordane (α -chlordane, γ -chlordane, heptachlor and its metabolite, heptachlor epoxide) were selected for quantitation in soils and plant material (Dorough *et al.*, 1969). However, no totally satisfactory method for quantitating technical chlordane residues has been devised.

Possibly, the need for developing such a method has been lessened. Velsicol Chemical Corp. recently developed a high purity chlordane reported to contain 95% or more of the α and γ isomers of chlordane (Velsicol Chem. Corp., 1970). This product is identified by Velsicol as HCS 3260, and is

used to identify the product in the text and tables of this paper.

Glc analysis of HCS 3260 in our laboratory showed that the product was comprised almost entirely of γ -chlordane, retention time 10.2 min, and α -chlordane, retention time 11.6 min (Figure 1). Since analytical reference standards for these two isomers are available, the problem of quantification one encountered with technical chlordane no longer applies. The effect of the purification of technical chlordane on its insecticidal effectiveness and residual persistence was evaluated in the current study.

METHODS AND MATERIALS

Treatment. Field plots (20 × 60 ft) of alfalfa (Narragansett variety), were selected for treatment with either technical chlordane or HCS 3260 sprays. The EC formulations, containing 8 and 4 lb of active ingredient (AI) per gal, respectively, were diluted with sufficient water to obtain treatment rates of 1 or 2 lb/acre (AI) when the crop was treated with 20 gal of spray per acre. Treatments were applied with a portable boom-type, compressed-air, hand sprayer equipped with four Spraying Systems 730308 TeeJet nozzles and operated at 30 psi. In the first experiment, applications were made on May 4, 1970, to alfalfa approximately 12 in. high. Identical applications were made to other alfalfa plots on September 11, 1970, when this crop was about 6 in. tall. Each treatment and the untreated check were replicated three times in a randomized complete block design. During the 21 days after the May treatment, the total rainfall was 1.8 in. and the average temperature was 19° C. For the same interval after the September treatment, there were 3.8 in. of rain and the average temperature was 23° C. The soil type in the treated area was Armor silt loam.

Sample Collection. Twenty soil cores, 1 × 4 in., were collected at random from each plot immediately before spraying the alfalfa and then 1 hr subsequent to the spraying operation. Additional soil samples were collected 21, 90, and 180 days after treatment. The soil cores from each plot were mixed thoroughly, placed in sealed containers, and frozen until analyzed.

The alfalfa was cut 21 days following treatment and representative samples of the "fresh cut" crop material were taken

*Department of Entomology, University of Kentucky, Lexington, Kentucky 40506.

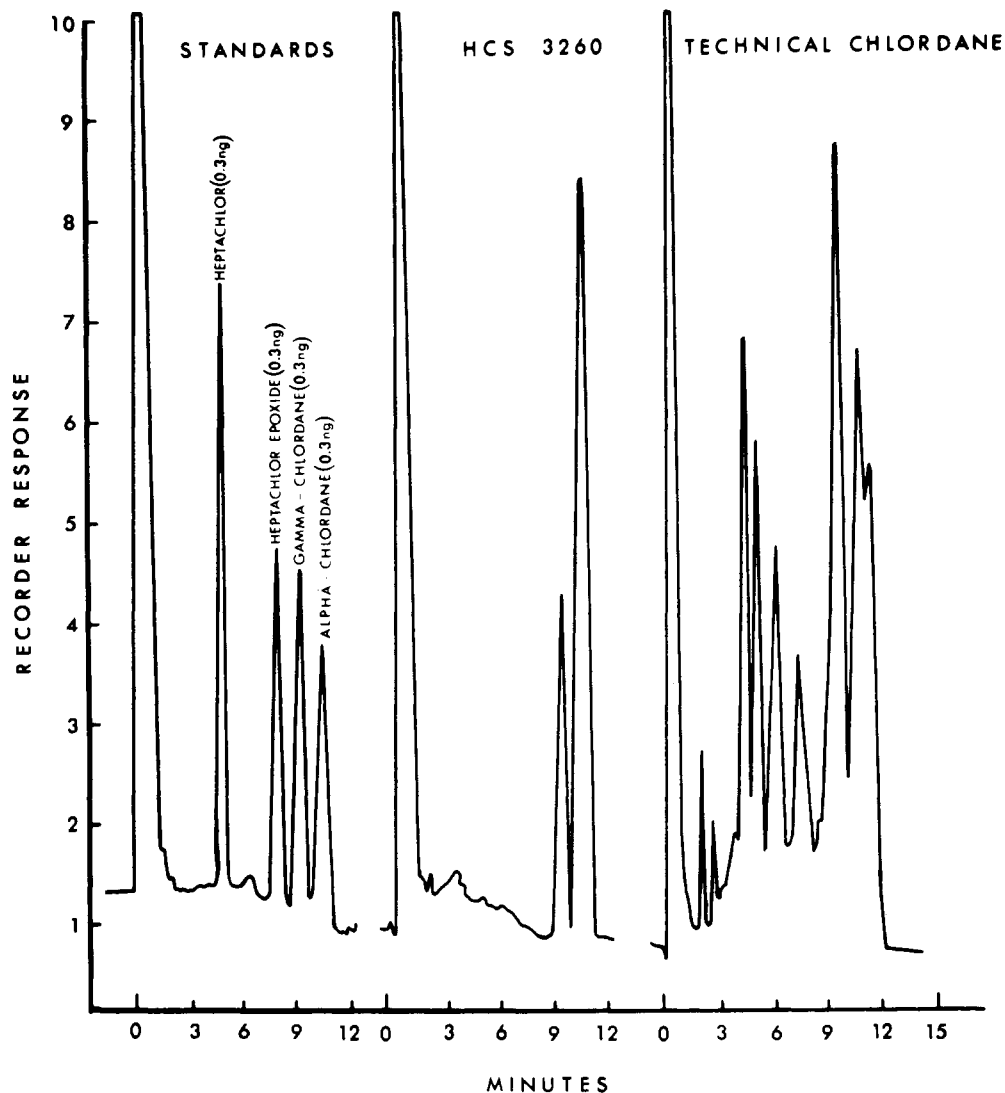


Figure 1. Gas chromatographic spectra of insecticide standards, HCS 3260 (2 ng of 4 lb/gal EC) and technical chlordane (5 ng of 8 lb/gal EC) on a DC-200 column. See methods section for complete operating parameters

from each plot and frozen immediately. The remainder of the cut alfalfa was allowed to remain in the field for 3 days before samples of the plants were collected and frozen. The latter samples are referred to as "field cured" alfalfa in the text and tables.

Prior to analysis, subsamples of the soils and alfalfa from each replication were oven-dried to a constant weight and the water content was calculated. No adjustment in the water content of field-collected samples was made for analysis; however, all data are expressed on the basis of the dry weight of the samples.

Extraction and Clean-up. All solvents used in these investigations were MC/B pesticide-quality materials. Insecticides were extracted from the alfalfa plants with acetonitrile. A 50-g sample was blended twice with the solvent, the volume being 300 ml and 150 ml, respectively, for the two extractions. Exactly one-half of the combined acetonitrile extracts, after filtering, was transferred to a 1-l. separatory funnel which contained 100 ml of petroleum ether and the mixture was shaken.

Five-hundred milliliters of a 2% sodium chloride solution were introduced into the funnel, the funnel was shaken thoroughly, and the phases were allowed to separate. The aqueous layer was again extracted with 100 ml of petroleum

ether and the ether extracts were combined and dried with anhydrous sodium sulfate. Next, the extract was filtered and concentrated to a volume of 2 ml for column cleanup.

A glass chromatographic column (20 mm i.d.), filled with 20 g of florasil, 60/100 mesh, and a 1-in. layer of anhydrous sodium sulfate, was prewashed with 10% diethyl ether in petroleum ether. The 2 ml extract of the alfalfa plants was added to the florasil column and the insecticides were eluted with 200 ml of the 10% diethyl ether solution. Normally, the eluate was concentrated to 10 ml for gas chromatographic analysis. When necessary to attain the desired sensitivity, the samples were further concentrated.

For extraction of the insecticides from soils, a 100-g sample was added to a quart jar containing 200 ml of a 1:1 mixture of benzene and acetone. The sample was then placed on an Eberbach flatbed shaker and shaken at 180 cpm for 1 hr. The solvent was decanted completely from the soil and passed through Whatman No. 1 filter paper into a 500-ml separatory funnel. A 2% sodium chloride solution, 200 ml, was then added and the contents were thoroughly shaken. After allowing the two phases to separate, the aqueous layer was extracted with 100 ml of benzene, which were then combined with the benzene from the initial soil extraction. The benzene was dried with anhydrous sodium sulfate, concentrated to a

volume of 2 ml, and subjected to a florasil column cleanup step as described for plant material. Aliquots of the concentrated eluate were injected into the gas chromatograph.

Gas Chromatographic Analysis. Each extract of soil or alfalfa was subjected to gas chromatographic analysis using Varian Aerograph Series 1700 instruments equipped with electron capture detectors. The following conditions were used.

	Column A	Column B
Column	Glass, 6 ft \times $\frac{1}{8}$ in. i.d.	5 ft \times $\frac{1}{8}$ in. i.d.
Packing	DC 200, 10% (w/w) on 80 to 90 mesh Anakrom ABS	SE 30, 3% (w/w) on 90 to 100 mesh Chromosorb P
Carrier gas	Nitrogen, 45 ml per min	Nitrogen, 25 ml per min
Temperatures	Column 195° C, injection port 200° C, detector 215° C	Column 200° C, injection port 200° C, detector 200° C

Recovery Tests. Analytical standards of α -chlordane, γ -chlordane, heptachlor, and heptachlor epoxide (Velsicol Chemical Corp., Chicago, Ill.) were used to determine the efficiency of the extraction procedures described above. Each material was added to the substrate, soil, or alfalfa at the 0.1-ppm level prior to the addition of the first extracting solvent. Recovery experiments were run on the individual insecticides and with a mixture of the four products. In addition to these recovery tests, the completion of extraction of aged residues from the soil and alfalfa was determined by re-extracting the solids after the normal procedure with the same and with different solvents, and analyzing these extracts separate from the original. Only when additional extractions failed to yield significant residues were the original procedures accepted.

Volatilization Experiments. The dissipation of α - and γ -chlordane from field-treated alfalfa was investigated under laboratory conditions. A series of samples of fresh cut alfalfa, collected 21 days after receiving a 1 lb/acre treatment of HCS 3260 on May 4, 1970, were spread evenly over a flat surface and allowed to remain at room temperature for a total of 14 days. At predetermined intervals, subsamples of the plant material were analyzed for pesticide and for moisture content.

To ascertain the nature of the escaped residues, similar samples of alfalfa were placed in containers designed so that air was passed over the plant material and into a cold hexane trap. The trap was operated for 8 hr and the nature and quantity of residues in the hexane were determined using the glc procedures already described.

Biological Activity. Surveys of the treated and untreated plots for alfalfa weevil larvae were made by taking 10 net sweeps/plot on 3, 7, 14, and 21 days after spraying the alfalfa on May 4, 1970. Also, an estimate of the insect damage was made by visual inspection of the crop and assigning a damage rating to each treatment. On the 21st day after treatment, the alfalfa was harvested and yield data were collected from the treated and untreated plots.

RESULTS AND DISCUSSION

Components of EC Formulations. Figure 1 shows that the technical chlordane was composed of at least nine components sensitive to electron capture detection. Although no attempt

was made to characterize each component, it was established that α -chlordane and γ -chlordane were present and that each accounted for approximately 14% of the total active ingredients. Heptachlor accounted for about 5% of the total active ingredients. The purified chlordane, HCS 3260, contained at least 74% α -chlordane and 24% γ -chlordane. By increasing the quantity of HCS 3260 injected into the glc by 100-fold that amount necessary for full scale deflection for the γ -chlordane peak, trace quantities of a material with the same retention time as heptachlor were noted. Its identity was not confirmed.

Considering the α and γ isomers as the only active ingredients in the EC formulations and calculating the lb/acre rates on a total AI basis revealed that applications of the HCS 3260 material resulted in five times more α -chlordane and two times more γ -chlordane than when the technical chlordane was applied. Thus, when 1 lb of AI/acre was desired, the actual α - + γ -chlordane applied was only 0.3 lb/acre in the case of technical chlordane but approximately the calculated rate of lb/acre for the HCS 3260. This is an important consideration in analyzing the data presented herein since residues reported in the soil and alfalfa were based only on the content of α -chlordane, γ -chlordane, heptachlor, and heptachlor epoxide. Total active ingredients on the technical chlordane-treated substrates were not quantitated.

One should keep in mind, then, that "total residues" means total active ingredients when referring to the HCS 3260 treated materials because the α and γ isomers were the only observed components of the AI. However, "total residues" in the technical chlordane treated soils and alfalfa refer only to the above stated compounds and their sum would have to be multiplied by a factor of approximately 3 to gain an estimate of the total "technical chlordane" present. This, of course, would require the assumption that all components of the technical chlordane (Figure 1) dissipated at the same rate. Detailed comparison of the 0-day and 21-day samples of soil and alfalfa showed that the major peaks generally maintained their relative peak heights even after considerable dissipation of the initial deposits had occurred. Therefore, this type of estimation of total technical chlordane residues very closely approximates the actual levels present in the substrates.

Recovery. The glc retention times of the four materials considered in the quantitation of residues in soil and alfalfa are shown in Table I. All of these materials were detectable

Table I. Glc Retention Time and Average Percentage Recoveries from Alfalfa and Soils of Chlordane and Related Compounds

Glc column ^a and substrate	Retention time, min, and average percent (\pm SE) recovery ^b			
	Heptachlor	Heptachlor epoxide	γ -chlordane	α -chlordane
DC 200 (10%)	5.4	8.7	10.2	11.6
SE 30 (3%)	7.1	11.7	12.7	14.0
Recovery from alfalfa	87 \pm 7	86 \pm 8	91 \pm 18	98 \pm 12
Recovery from soils	94 \pm 10	89 \pm 11	85 \pm 10	89 \pm 8

^a Glc column and operating parameters given in methods. ^b Samples fortified at the 0.1-ppm level prior to extraction.

Table II. Residues in Soils after Field Treatments of Alfalfa in May with EC Formulations of Chlordane and HCS 3260^a

Treatment and elapsed time ^c	Average ppm (\pm SE) at indicated rate (dry wt) ^b					
	α -Chlordane		γ -Chlordane		Total	
	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre
1 hr (25.7)						
Chlordane	0.004 \pm 0.002	0.013 \pm 0.006	0.002 \pm 0.001	0.011 \pm 0.006	0.006 \pm 0.003	0.024 \pm 0.011
HCS 3260	0.024 \pm 0.006	0.027 \pm 0.012	0.009 \pm 0.003	0.010 \pm 0.008	0.033 \pm 0.009	0.037 \pm 0.016
21 days (24.5)						
Chlordane	0.005 \pm 0.002	0.008 \pm 0.001	0.004 \pm 0.002	0.007 \pm 0.001	0.009 \pm 0.003	0.015 \pm 0.003
HCS 3260	0.021 \pm 0.003	0.055 \pm 0.003	0.007 \pm 0.001	0.019 \pm 0.009	0.028 \pm 0.004	0.074 \pm 0.035
90 days (22.2)						
Chlordane	0.007 \pm 0.004	0.010 \pm 0.003	0.005 \pm 0.003	0.008 \pm 0.002	0.012 \pm 0.006	0.018 \pm 0.005
HCS 3260	0.022 \pm 0.020	0.034 \pm 0.010	0.006 \pm 0.002	0.016 \pm 0.004	0.028 \pm 0.009	0.050 \pm 0.013
180 days (27.0)						
Chlordane	0.066 \pm 0.004	0.010 \pm 0.007	0.003 \pm 0.001	0.009 \pm 0.006	0.009 \pm 0.006	0.019 \pm 0.006
HCS 3260	0.022 \pm 0.010	0.042 \pm 0.034	0.006 \pm 0.003	0.012 \pm 0.009	0.028 \pm 0.013	0.054 \pm 0.044

^a Alfalfa, 12 in. high, treated with technical chlordane or HCS 3260, a high purity chlordane containing 95% or more of α - and γ -chlordane, in a total spray volume of 20 gal/acre. Treated May 4, 1970. ^b No heptachlor or heptachlor epoxide (<0.001 ppm) detected in these soils. Data are averages from three field replications. ^c Numbers in parentheses indicate the average percent of water in samples.

Table III. Residues in Soils after Field Treatments of Alfalfa in September with EC Formulations of Technical Chlordane and HCS 3260^a

Treatment and elapsed time ^c	Average ppm (\pm SE) at indicated rate (dry wt) ^b					
	α -Chlordane		γ -Chlordane		Total	
	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre	1 lb/acre	2 lb/acre
1 hr (15.4)						
Chlordane	0.053 \pm 0.001	0.105 \pm 0.015	0.049 \pm 0.002	0.100 \pm 0.013	0.102 \pm 0.003	0.205 \pm 0.029
HCS 3260	0.238 \pm 0.043	0.383 \pm 0.147	0.084 \pm 0.020	0.172 \pm 0.038	0.322 \pm 0.062	0.555 \pm 0.181
21 days (21.7)						
Chlordane	0.024 \pm 0.003	0.040 \pm 0.014	0.024 \pm 0.003	0.041 \pm 0.016	0.048 \pm 0.008	0.081 \pm 0.030
HCS 3260	0.135 \pm 0.010	0.199 \pm 0.048	0.055 \pm 0.005	0.077 \pm 0.020	0.190 \pm 0.011	0.276 \pm 0.068
90 days (23.8)						
Chlordane	0.015 \pm 0.003	0.024 \pm 0.004	0.013 \pm 0.002	0.021 \pm 0.004	0.028 \pm 0.005	0.045 \pm 0.007
HCS 3260	0.055 \pm 0.005	0.103 \pm 0.030	0.024 \pm 0.004	0.035 \pm 0.013	0.079 \pm 0.009	0.138 \pm 0.043
180 days (25.8)						
Chlordane	0.004 \pm 0.001	0.009 \pm 0.003	0.005 \pm 0.001	0.010 \pm 0.004	0.009 \pm 0.001	0.019 \pm 0.007
HCS 3260	0.044 \pm 0.020	0.064 \pm 0.017	0.018 \pm 0.007	0.028 \pm 0.006	0.062 \pm 0.027	0.092 \pm 0.023

^a Soils from alfalfa, 6 in. high, plots sprayed with technical chlordane or HCS 3260 in a total volume of 20 gal/acre. HCS 3260 is a high purity chlordane containing 95+% α - and γ -chlordane. Treated September 11, 1970. ^b No heptachlor or heptachlor epoxide (<0.001 ppm) detected in these soils. Data are averages from three field replications. ^c Numbers in parentheses indicated average percent of water in soils.

at very low levels using the electron capture detector (Figure 1). With control samples, the levels of interfering materials in the soils and alfalfa were sufficiently low so that sensitivity levels of 0.001 and 0.004 ppm, respectively, were easily attainable.

Average recoveries of α - and γ -chlordane heptachlor, and heptachlor epoxide from soil and alfalfa were 85% or better. Variations in the percentage recovery of the different compounds were considerable (Table I), but do represent the situation as it occurred over a 4-month period of analysis since each set of samples analyzed daily included at least one recovery experiment.

Soils. Insecticide residues reaching the soil from the treatment of alfalfa in May consisted only of α - and γ -chlordane and were about $1/10$ those in the soil when the crop was sprayed in September (Tables II and III). This probably resulted because of the increased ground cover provided by the alfalfa in the first treatment as compared to that in the second. As noted earlier, the plants sprayed in May were approximately 1 ft in height while those sprayed in September

were about one-half that height. Maximum residues, 0.6 ppm, were deposited on the soils sprayed in September with 2 lb/acre of HCS 3260 (Table III). When calculated on a 4-in. acre basis, this accounted for 40% of the applied AI.

Dissipation of the α - and γ -chlordane occurred very slowly, if at all, during the 180-day period following the treatments in May. Factors contributing to such slow disappearance of the pesticides were the rapid regrowth of plants following harvest 21 days after treatment, very little rainfall, and a rather low average daily temperature. In the second test, the level of residues dropped about 50% during the first 21 days after treatment in September. The level of residues continued to decline and approximated those from the May treatment when analysis of each was conducted on samples collected 180 days after treatment. The more rapid dissipation of residues from the soil after the second treatment was likely facilitated by the higher temperatures and greater rainfall than occurred in the experiment initiated in May.

Alfalfa. There was excellent agreement of the data relative to the level of residues on alfalfa 21 days following treatment

Table IV. Residues in Alfalfa 21 Days after Treatments in May with EC Formulations of Technical Chlordane and HCS 3260 at Rates of 1 and 2 lb/acre^a

Treatment and sample ^c	Average ppm (\pm SE) in fresh cut and field-cured alfalfa (dry wt) ^b				Total
	α -Chlordane	γ -Chlordane	Heptachlor	Heptachlor epoxide	
1 lb per acre					
Fresh cut (77.8)					
Chlordane	1.32 \pm 0.56	1.08 \pm 0.64	<0.001	0.25 \pm 0.09	2.65 \pm 1.30
HCS 3260	4.11 \pm 1.31	1.35 \pm 0.31	<0.001	<0.001	5.46 \pm 1.33
Field-cured (30.5)					
Chlordane	0.39 \pm 0.03	0.31 \pm 0.08	<0.001	0.09 \pm 0.02	0.79 \pm 0.09
HCS 3260	2.24 \pm 0.84	0.71 \pm 0.25	<0.001	<0.001	2.95 \pm 1.08
2 lb per acre					
Fresh cut (77.8)					
Chlordane	2.03 \pm 1.02	1.99 \pm 1.11	<0.001	0.52 \pm 0.35	4.54 \pm 2.55
HCS 3260	11.72 \pm 4.10	3.72 \pm 2.78	<0.001	<0.001	15.44 \pm 11.37
Field-cured (30.5)					
Chlordane	0.60 \pm 0.11	0.64 \pm 0.15	<0.001	0.16 \pm 0.08	1.40 \pm 0.32
HCS 3260	2.54 \pm 1.58	0.68 \pm 0.55	<0.001	<0.001	3.22 \pm 2.33

^a Alfalfa, 12 in. high, treated with 20 gal/acre technical chlordane spray or with HCS 3260 spray, a high purity chlordane containing 95+% α - and γ -chlordane. Treated May 4, 1970. ^b "Fresh cut" samples were frozen immediately after cutting. "Field-cured" samples remained in the field for 3 days before freezing. Data are averages from three field replications, two analyses per replication. ^c Numbers in parentheses indicate average percent of water in samples.

Table V. Residues on Alfalfa 1 Hour and 21 Days Following Treatments in September with EC Formulations of Chlordane and HCS 3260 at Rates of 1 and 2 lb/acre^a

Treatment and elapsed time ^c	Average ppm (\pm SE) in fresh cut and field-cured alfalfa (dry wt) ^b				Total
	α -Chlordane	γ -Chlordane	Heptachlor	Heptachlor epoxide	
1 lb per acre					
1 hr, fresh cut (76.3)					
Chlordane	13.64 \pm 1.63	13.91 \pm 2.04	1.77 \pm 0.06	<0.001	29.32 \pm 3.73
HCS 3260	96.56 \pm 6.36	33.39 \pm 6.83	0.17 \pm 0.04	<0.001	130.12 \pm 12.90
21 days, fresh cut (76.0)					
Chlordane	0.76 \pm 0.08	0.71 \pm 0.07	<0.001	0.23 \pm 0.04	1.70 \pm 0.17
HCS 3260	5.60 \pm 0.40	1.75 \pm 0.37	<0.001	<0.001	7.35 \pm 1.12
21 days, cured (16.3)					
Chlordane	0.39 \pm 0.04	0.40 \pm 0.01	<0.001	0.15 \pm 0.02	0.94 \pm 0.08
HCS 3260	2.16 \pm 0.78	0.61 \pm 0.07	<0.001	<0.001	2.77 \pm 0.80
2 lb per acre					
1 hr, fresh cut (76.3)					
Chlordane	34.23 \pm 4.74	35.35 \pm 4.84	6.88 \pm 1.18	<0.001	76.46 \pm 10.45
HCS 3260	453.72 \pm 68.91	163.28 \pm 15.53	0.65 \pm 0.11	<0.001	617.65 \pm 72.39
21 days, fresh cut (76.0)					
Chlordane	1.26 \pm 0.27	1.34 \pm 0.54	0.05 \pm 0.01	0.38 \pm 0.09	3.03 \pm 0.87
HCS 3260	18.60 \pm 1.84	5.79 \pm 0.75	<0.001	<0.001	24.39 \pm 2.69
21 days, cured (16.3)					
Chlordane	0.61 \pm 0.24	0.57 \pm 0.22	0.17 \pm 0.07	<0.001	1.35 \pm 0.51
HCS 3260	8.15 \pm 2.54	2.51 \pm 1.23	<0.001	<0.001	10.66 \pm 4.18

^a Alfalfa, 6 in. high, treated with 20 gal/acre technical chlordane spray or HCS 3260 spray, a high purity chlordane containing 95+% α - and γ -chlordane. Treated September 11, 1970. ^b "Fresh cut" samples were collected immediately after cutting and frozen until analyzed. "Field-cured" samples were allowed to remain in the field for 3 days after cutting and then frozen until analyzed. Data are averages from three field replications, two analyses per replication. ^c Numbers in parentheses represent the average percent of water in samples.

Table VI. Comparative Concentrations of α - and γ -Chlordane in EC Formulations of Technical Chlordane and HCS 3260 and in Alfalfa Sprayed with These Insecticides

Sample ^b	Ratios ^a				
	T α /T γ	H α /H γ	H α /T α	H γ /T γ	H α + γ / T α + γ
EC formulations					
Alfalfa, 1 hr, fresh cut, 1 lb/acre	1.0	2.6	6.0	2.2	4.0
Alfalfa, 21 day, fresh cut, 1 lb/acre	1.0	2.9	7.1	2.3	4.7
Alfalfa, 21 day, field-cured, 1 lb/acre	1.1	3.2	7.4	2.5	5.0
Alfalfa, 21 day, field-cured, 2 lb/acre	1.0	3.5	5.5	1.5	3.5

^a T = technical chlordane, 8 lb AI per gal. H = HCS 3260, 4 lb AI per gal. α = alpha-chlordane; γ = gamma-chlordane. ^b Data pertaining to alfalfa taken from Table V.

Table VII. Loss of Residues at Room Temperature from Freshly Cut Alfalfa Sprayed 21 Days Prior to Cutting with 2 lb/acre of HCS 3260^a

Residue	Initial ppm	Percent loss of residues after indicated days ^b		
		3	7	14
α -Chlordane	4.22	57.4	63.2	62.0
γ -Chlordane	1.10	51.6	59.1	68.4
Total	5.32	52.8	61.0	67.2

^a HCS 3260, a high purity chlordane containing 95+% α - and γ -chlordane. ^b Ppm residues at each time interval were calculated on the basis of the original weight of the sample (50 g) although the weight of the sample was reduced 70% in 3 days as a result of water loss. No further loss was noted after 3 days.

Table VIII. Comparative Effect of Technical Chlordane and HCS 3260 Treatment on Alfalfa Weevil Control and on Alfalfa Yields^a

Treatment and rate	Larvae numbers (A) and damaging rating (B) at indicated days after treatment ^b								Yields, lb dry weight ^c
	3		7		14		21		
	A	B	A	B	A	B	A	B	
1.0 lb/acre									
Chlordane	5.7		9.0	2.7	143.0	4.3	552.6	6.0	17.3 yz
HCS 3260	2.3		3.3	3.0	130.0	2.7	383.3	5.3	18.3 yz
2.0 lb/acre									
Chlordane	4.3		3.0	2.8	117.3	3.7	366.6	5.0	16.5 xy
HCS 3260	4.4		1.3	2.5	75.3	2.3	265.3	4.3	19.5 z
Control	64.7		140.3	3.7	398.0	7.0	650.0	8.0	14.4 x

^a HCS 3260, a high purity chlordane containing 95+ % α - and γ -chlordane. Applications of EC formulations made May 4, 1970, Lexington, Kentucky. All data are averages of three field replications. ^b Larvae per ten sweeps. Damage rating based on a 1-9 scale where 1 indicates no damage and 9 indicates complete skeletonization. ^c Averages of 10 ft \times 20 ft plots from each replication. Yield data collected 21 days after treatment. Treatment means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

in May and in September (Tables IV and V). In fact, the differences observed after the two different treatments were no greater than that variation experienced with the different replications within the same experiment. The data indicated that 21-day fresh cut alfalfa contained 2-3 ppm of the assayed residues when sprayed with 1 lb/acre of technical chlordane and 5-8 ppm when treated with the purified chlordane, HCS 3260. Based on the second test (Table V), this represents a 94% reduction in residues from the time of insecticide application (1 hr, fresh cut) until the time of harvest 21 days later (21 day, fresh cut). That the losses did not involve the selective dissipation of either α - or γ -chlordane, or that the "impurities" in technical chlordane did not appreciably effect the residual nature of these isomers is demonstrated by the data in Table VI. Ratios of the two isomers in the EC formulations of the insecticides compared very favorably with the same ratios observed on the treated alfalfa. With the HCS 3260 treated plants, the residues on the 21-day samples were composed entirely of α - and γ -chlordane. Small amounts of heptachlor epoxide were detected on comparable samples of the technical chlordane-treated alfalfa; occasionally, traces of a compound with a retention time the same as that of heptachlor also were observed.

Significant losses of insecticide residues occurred when the alfalfa plants were allowed to weather in the field for 3 days. There were reductions in all detected components of the residues and, while there was considerable variation, there did not appear to be a great selective loss of a particular residue during the process. By averaging the amount of loss by all samples in the first test and those in the second, it was found that weathering for 3 days reduced the level of residues by 56% in the May experiment and by 58% in the September test.

Volatilization of Residues. Fresh cut alfalfa containing α - and γ -chlordane residues (Table V, 1 lb/acre, HCS 3260) lost about 50% of those residues when let stand for 3 days at room temperature (Table VII). Thereafter, the rate of dissipation proceeded at a very slow rate, with a total loss in 14 days of 60 to 70%. The very rapid loss during the first 3 days corresponded to the loss of moisture from the samples. Although this indicated that the reduction of chlordane residues involved the loss of water, further experimentation showed that water loss was not required in order to get a substantial decline in the level of these residues on alfalfa. It was found, for example, that oven-dried alfalfa continued to lose residues even though there was no further decrease in moisture content of the plant material. Analysis of a hexane trap demonstrated that α - and γ -chlordane were volatilized intact from the alfalfa.

Biological Activity. Field evaluations of both technical chlordane-treated and HCS 3260-treated alfalfa showed that the purified chlordane was equal to or better than the technical chlordane for alfalfa weevil control (Table VIII). At comparable rates, the number of alfalfa weevil larvae collected from the plots was lower when HCS 3260 was used. Consequently, the degree of insect damage to the plants was slightly less. The final criterion of comparative effectiveness, that of alfalfa yield, was indiscernible. Both chlordane sprays yielded significantly more plant material than plots which were not treated, yet any real advantage of one material over the other was not demonstrated.

The improvement in the synthesis of technical chlordane to yield a product (HCS 3260) that was 98+ % α - and γ -chlordane serves as an excellent example of what can and should be done with a number of compounds currently considered commercial successes. Impurities in a pesticide, or a multi-component product, complicate the toxicological evaluations of a product and may result in the misinterpretation of those data which are obtained. This could create unnecessary health hazards and/or force an otherwise desirable product from the market. It is advisable, then, that the purity of all pesticides be critically evaluated and that the significance of any impurity in the biological activity of the material be clearly defined. With chlordane this has been largely accomplished. Over 98% of the applied AI of HCS 3260 consists of two known products which may be quantitated by common glc residue methods. Now, the fate of the high-purity chlordane when applied under practical conditions can be critically evaluated. This was virtually impossible with technical chlordane since about 65% of the AI was undefined as to chemical identity and consisted of five or more compounds.

ACKNOWLEDGMENT

The authors are grateful to the Velsicol Chemical Corp., Chicago, for supplying the insecticides and analytical standards used in this study.

LITERATURE CITED

- Dorough, H. W., Randolph, N. M., Teetes, G. W., Tex. Agr. Exp. Sta. MP-936, Nov 1969, 6 pp.
 Ingle, I., "A Monograph on Chlordane," University of Ill., Urbana, 1965, 88 pp.
 Klein, A. X., Link, J. D., *J. Ass. Offic. Agr. Chem.* **50**, 586 (1967).
 Martin, H., "Pesticide Manual," 1st ed., British Crop Protection Council, 1968, p 80.
 Thurston, A. D., *J. Ass. Offic. Agr. Chem.* **48**, 952 (1965).
 Velsicol Chemical Corporation, Chicago, Ill., Bulletin No. EI-1-3, 1970.

Received for review April 27, 1971. Accepted June 28, 1971. This work was supported in part from funds from the Velsicol Chemical Corp. and from Regional Research Project S-73.