Decline and Movement of AG Chlordane in Soil and Its Residues in Alfalfa

Francesco Tafuri,* Mario Businelli, Luciano Scarponi, and Cesare Marucchini

Analytical methods for the determination of α-, γ-, oxy-, and photo-cis-chlordane are described. Experimental plots were treated prior to planting with AG chlordane 10% granules at dose rates of 3 and 6 kg of AI/ha. Alfalfa was sown within 1 h from when AG chlordane had been incorporated into the soil to a depth of 10 cm. A high proportion of the γ-chlordane originally applied persisted in the soil at the time of the second cutting, 169 days after application, thereby showing that γ-chlordane is more stable than the α isomer. Insecticide movement through the soil was considerable. The difference between the quantities of α- and γ-chlordane recovered in the analysis from washed and unwashed alfalfa would seem to suggest that an appreciable part is present on the plant surface, possibly in consequence of contamination by particles of treated soil. However, surface contamination is considerably less than the quantity which penetrates into the plant tissues, although the total amount recovered from washed plants is relatively small. Photo-cis-chlordane residues in alfalfa were always below the sensitivity limit (0.001 ppm), while oxychlordane, found only in the second cutting, gave values of 0.001 and 0.002 ppm at dose levels of 3 and 6 kg of AI/ha.

AG chlordane, recently developed by the Velsicol Chemical Corp., contains about 95% octachloro-4,7-methanotetrahydroindene, made up of about 70% of the α (or cis) isomer and 25% of the γ (or trans) isomer and less than 1% of heptachlor. The old technical chlordane, used commercially for over 35 years, contained no more than 60% of the chlordane α and γ isomers but up to 10% of heptachlor (Melnikov, 1971; Martin, 1974; Furness, 1971).

In animals, almost all chlordane α and γ isomers are converted to water-soluble metabolites which are rapidly excreted. A small part, however, is metabolized via oxychlordane to the lipophilic 1,2-dichlorochlordene epoxide (Dorough and Hemken, 1973). As neither chlordane nor its metabolites should be accumulated in the food chain (Furness, 1971), the new low heptachlor content chlordane is of great interest. Model ecosystem studies show that the environmental behavior of heptachlor is somewhat like that of aldrin in its rapid epoxidation to heptachlor epoxide. The epoxidation of heptachlor is an intoxication reaction producing the substantially more toxic heptachlor epoxide which is very persistent in the environment and bioaccumulative in plant and animal tissues (PoYung et al., 1975). Although AG chlordane is a less powerful insecticide than heptachlor at a corresponding dose, it is, nevertheless, effective against wireworms (*Agriotes* spp.) and spotted millipede (*Blaniulus guttulatus*) in the soil (Furness, 1971; Kovacs and Maini, 1972; Kovacs et al., 1973; L'Hotellier, 1973).

For these reasons, experiments were undertaken to determine the residues of chlordane α and γ isomers and their metabolites in alfalfa which had been seeded within 1 h of applying AG chlordane to the soil. In addition to oxychlordane, which can appear as a residue in plants (Wilson and Oloffs, 1973), we assayed for photo-cis-chlordane, a photoisomer of α-chlordane. In order to study the surface concentration and possible penetration of the insecticide, attempts were made to wash the alfalfa crop before preparing it for residue analysis. A further objective of the reported experiments was to determine the persistence of AG chlordane in the soil after broadcast application in quantities similar to those used in farming practice.

Centro di Studio sulla Chimica degli Antiparassitari del C.N.R., Istituto di Chimica Agraria dell'Università, 06100 Perugia, Italy.

Table I. Properties of the Clay Loam Soil

Organic matter, %	1.7
pH	7.9
Mechanical anal., g/kg	
Sand	352
Silt	362
Clay	286

EXPERIMENTAL SECTION

Field Trials. The experiments were laid out on a clay loam soil in the province of Perugia; some of the revelant properties of this soil are shown in Table I. The experimental areas consisted of nine plots each 10 × 10 m with 1 m between the plots.

On April 14, 1975, AG chlordane (10% granules) was applied at the rates 3 and 6 kg of AI/ha; three plots were treated with each dose. The insecticide was immediately disked into the soil to an average depth of 10 cm. Three plots were left untreated, the position of plots being randomized within the experiment. Alfalfa seeds (local variety, 40 kg/ha) were sown in rows 15 cm apart on all plots within 1 h of insecticide application. From sowing time onward meteorological data were recorded. The agricultural practices followed on our experimental plots corresponded closely to the practices applied to the growing of alfalfa in the Perugia area. The alfalfa seedlings had emerged by May 2. On July 2, 1975 alfalfa was cut for the first time and samples of the soil were taken at depths of 0-10 cm and 10-30 cm. On Sept 30, 1975 alfalfa was cut for the second time and once again samples of the soil were collected at 0-10 cm and 10-30 cm. Nine soil cores (2.5-cm diameter, 30 cm deep) were taken from each plot and these were divided into upper (0-10 cm) and lower (10-30 cm) sections; all samples from the same plot and stratum were bulked to obtain samples as representative as possible. Representative samples of first and second cutting alfalfa forage were taken from each plot. Samples were stored at -24 °C until analysis. AG chlordane residues were determined both in unwashed alfalfa and in alfalfa washed with 1% lauryl sulfate solution.

ANALYTICAL METHODS

Extraction of the Insecticide from Soil Samples. Air-dried soil (100 g) was passed through a 0.75-mm sieve and placed in a 500-ml glass-stoppered flask. Distilled water (30 ml) and a 1:1 mixture of n-hexane + 2-propanol (300 ml) were added. This mixture was agitated for 30 min and centrifuged at 6000 rpm for 15 min, and then the solution was withdrawn. The sample was washed with

Table II. Mean Percentage Recoveries of α- and γ-Chlordane and Their Metabolites Added to Soil and Alfalfa Tissue before Extraction, ± Standard Errors for n Determinations

Amts added, mg/kg	Soil		Alfalfa			
	α	γ	α	γ	Oxy	Photo-cis
0.001			114 ± 1.9 (7)	118 ± 2.1 (7)	110 ± 2.3 (7)	80 ± 1.8 (7)
0.002			107 ± 1.4 (5)	98 ± 1.8 (5)	116 ± 2.1 (5)	82 ± 1.9 (5)
0.005	78 ± 2.2 (6)	101 ± 1.9 (5)	109 ± 1.5 (6)	104 ± 2.2 (6)	89 ± 1.9 (5)	83 ± 1.5 (5)
0.010	85 ± 1.8 (5)	90 ± 2.3 (4)	92 ± 1.6 (5)	81 ± 1.4 (4)	85 ± 1.9 (5)	78 ± 1.7 (5)
0.020	83 ± 2.3 (4)	84 ± 1.6 (5)	85 ± 1.8 (5)	80 ± 1.7 (5)	112 ± 2.0 (5)	94 ± 1.3 (6)
0.500	95 ± 1.5 (5)	88 ± 1.8 (4)				
2.000	102 ± 1.7 (4)	92 ± 2.1 (3)				

extracting solution (100 ml) and then centrifuged. The extracts were combined and extracted with distilled water (3 × 100 ml) in a separating funnel. The aqueous phases were combined and extracted twice with n-hexane (2 × 25 ml) and then discarded. The combined organic solvent extracts were dried with anhydrous sodium sulfate and utilized directly for gas chromatographic determination. If necessary, an aliquot of the extract was concentrated using a rotary evaporator at 35 °C.

Extraction of the Insecticide from Alfalfa. Chopped vegetable matter (20 g) was treated in a blender jar and homogenized with successive aliquots of ethyl acetate; the filtered extracts were diluted to 200 ml with ethyl acetate. An aliquot (50 ml) of this ethyl acetate solution was concentrated to 2–3 ml, using a rotary evaporator at 35 °C. The concentrated extract was injected a number of times, using nitrogen as the carrier gas, onto a column of silanized glass beads, heated to 180 °C, in a Sweep-Co-Distiller K-500 750 (Kontes Co., Vineland, N.J.). The volatilized solvent containing the pesticide, which had been separated from nonvolatile constituents, was condensed in a coil immersed in a bath at 0 °C. It was then evaporated to dryness with a gentle stream of dry nitrogen. The residue was dissolved in 3 ml of pentane and then passed through a Florisil column, employing the following procedure: 3 g of 60/100 PR grade Florisil (Floridin Co.) was activated at 250 °C for 150 min and then introduced into a column as a slurry in pentane. After packing, the pentane solution of plant extract was applied to the top of the column. Twenty milliliters of pentane followed by 20 ml of a 5 + 1 mixture of benzene + pentane were used to elute the insecticide from the column. The eluate was evaporated to dryness in a rotary evaporator at 35 °C. The residue was taken up with 5 ml of pentane, aliquots (20–30 μl) of which were injected into the gas chromatograph.

Gas Chromatography. A Perkin-Elmer Model 900 gas chromatograph equipped with an electron-capture detector ([63]Ni source) and a Hitachi–Perkin-Elmer 196 1-mV recorder were employed. Two glass columns were used: I (1.80 m × 6 mm) for the α and γ isomers and oxychlordane determinations contained 80–100 mesh Chromosorb WHP coated with SE 30 (2% w/w) plus QF 1 (6% w/w) (Wilson and Oloffs, 1973); and II (1.22 m × 6 mm) for the photo-cis-chlordane determination was packed with 80–100 mesh Chromosorb WHP coated with OV 1 (3% w/w). Injections were made with 10- or 50-μl Hamilton syringes. Chromatographic runs were performed isothermally and the column temperature was maintained at 190 °C, injection port at 250 °C, and detector at 200 °C. Argon with 4.5% methane was used as carrier gas at 27.3 ml/min and as scavenging gas at 55.7 ml/min. The chart speed was 1 cm/min. α and γ isomers, oxychlordane, and photo-cis-chlordane were estimated on the basis of peak area (peak height × the width at half-peak height). The calibration curve of peak area × picograms of each compound, using analytical reference compounds as standards, was

plotted on linear graph paper. Each point of this curve represented the average of five determinations. Retention times, on column I, were 12 min, 12 s for oxychlordane, 14 min, 36 s for γ-chlordane, and 16 min, 12 s for α-chlordane, and, on column II, 7 min, 18 s for photo-cis-chlordane.

Peak areas corresponding to an injection of 10 pg, at attenuation ×1, were the following: 16.5 cm² for oxychlordane, 25.6 cm² for γ isomer, 16.2 cm² for α isomer, and 13.4 cm² for photo-cis-chlordane.

RESULTS AND DISCUSSION

To determine percentage recovery, various known quantities of α and γ isomers were added in solvent to soil and alfalfa; oxy- and photo-cis-chlordane were added only to alfalfa. The solvent was evaporated and the samples were then analyzed as described above. The efficiency of the analytical methods is indicated by the recovery of the α and γ isomers and oxy- and photo-cis-chlordane added to untreated samples (Table II). In soil, the recoveries varied from 78 to 102% for α- and from 84 to 101% for γ-chlordane; in alfalfa from 85 to 114% for α isomer, from 80 to 118% for γ isomer, from 85 to 116% for oxychlordane, and from 78 to 94% for photo-cis-chlordane. The recoveries were satisfactory in relation to the concentration levels tested. The percentage recovery ranges from alfalfa for α and γ isomers, reported here, are wider than reported in our previous work (Tafuri, et al., 1974). The difference, apart from the lower concentrations tested, is due to the necessary changes in extraction and purification methods for determining oxy- and photo-cis-chlordane. Sweep codistillation as a purification process for routine determination of organochlorine pesticide residues (Gay and Cerny, 1975) has yielded good results. Taking into account the chromatograms of the numerous blanks tested (9 from soils, 8 from alfalfa), the limits of sensitivity were 0.001 ppm.

Figure 1 shows the ambient temperature and rainfall during the period of the experiment. Residues of α- and γ-chlordane found in soil samples at depths of 0–10 cm and 10–30 cm are reported in Table III.

The possibility of analytical error in determining the quantities of α and γ isomers in soil samples from the untreated plots should be discounted because the specificity of the analytical procedure had been thoroughly tested. In previously reported corresponding experiments, analyses have indicated that samples of soil from untreated control plots contained α- and γ-chlordane (Furness, 1971; Tafuri et al., 1973–1974).

Residue data at 0 days after insecticide incorporation show that AG chlordane was uniformly distributed in the 0–10-cm layer. The chlordane residue concentrations which were found agree well with that expected from an arithmetical approach. If the bulk density of soil at Perugia = 1.20 g/cm³ and 70% of the α isomers and 25% of the γ isomers in the AG chlordane are assumed, the

Table III. Residues (mg/kg) of α- and γ-Chlordane in Soil Samples at Two Different Depths (0-10 and 10-30 cm) 0, 79, and 169 Days after Insecticide Application

Soil sampling date	Untreated				3 kg of AI/ha				6 kg of AI/ha			
	0-10 cm		10-30 cm		0-10 cm		10-30 cm		0-10 cm		10-30 cm	
	α	γ	α	γ	α	γ	α	γ	α	γ	α	γ
April 14, 1975	0.136	0.006	ND[a]	ND	1.863	0.612	ND	ND	3.794	1.290	ND	ND
July 2, 1975	0.090	0.005	ND	ND	0.448	0.175	0.334	0.154	1.555	0.581	1.019	0.438
Sept 30, 1975	0.088	0.004	ND	ND	0.627	0.275	0.190	0.075	1.261	0.625	0.532	0.342

[a] ND = nondetectable.

Table IV. α- and γ-Chlordane (mg/kg) 0, 79, and 169 Days after Insecticide Application Observed at the Two Different Depths (0-10 and 10-30 cm) Considered as Still All Ideally Present in the Upper 0-10-cm Layer of the Soil[a]

Soil treatment with AG chlordane, kg of AI/ha	April 14, 1975		July 2, 1975		Sept 30, 1975		Means	
	α	γ	α	γ	α	γ	α	γ
3	1.863	0.612	1.116	0.483	1.007	0.425	1.329A	0.507a
6	3.794	1.290	3.593	1.457	2.325	1.309	3.237B	1.349b
Means	2.829aA	0.951a	2.355bAB	0.965a	1.666cB	0.867a		

[a] Means followed by the same letter are not significantly different (small letter at $p = 0.05$, capital letter at $p = 0.01$).

Table V. Average Residues (μg/kg) of α-Chlordane in Unwashed and Washed Alfalfa at Each of Two Dates of Cutting[a]

Soil treatment with AG chlordane, kg of AI/ha	First cut (July 2, 1975)		Second cut (Sept 30, 1975)		Means				
	Unwashed	Washed	Unwashed	Washed	Application rates	Unwashed	Washed	First cut	Second cut
0	2.667	1.333	3.000	1.667	2.167A	2.833	1.500	2.000	2.333
3	9.667	7.333	5.667	4.000	6.667B	7.667	5.667	8.500	4.833
6	10.333	7.000	5.667	5.333	7.083B	8.000	6.167	8.667	5.500
Means	7.556	5.222	4.778	3.667	5.305	6.167A	4.444B	6.389a	4.222a

[a] Means followed by the same letter are not significantly different (small letter at $p = 0.05$, capital letter at $p = 0.01$).

Table VI. Average Residues (μg/kg) of γ-Chlordane in Unwashed and Washed Alfalfa at Each of Two Dates of Cutting[a]

Soil treatment with AG chlordane, kg of AI/ha	First cut (July 2, 1975)		Second cut (Sept 30, 1975)		Means				
	Unwashed	Washed	Unwashed	Washed	Application rates	Unwashed	Washed	First cut	Second Cut
0	1.000	1.000	2.000	1.667	1.417a	1.500	1.333	1.000	1.833
3	4.333	2.667	4.667	2.667	3.583b	4.500	2.667	3.500	3.667
6	3.000	2.333	5.000	4.667	3.750b	4.000	3.500	2.667	4.833
Means	2.778	2.000	3.889	3.000	2.917	3.333a	2.500b	2.389a	3.444b

[a] Means followed by the same letter are not significantly different (small letter at $p = 0.05$, capital letter at $p = 0.01$).

calculated concentrations would be the following: for dose 3 kg of AI/ha, 1.750 ppm of α-chlordane and 0.625 ppm of γ-chlordane; for dose 6 kg of AI/ha, 3.500 ppm of α-chlordane and 1.250 ppm of γ-chlordane.

It is very important to consider the concentrations of α- and γ-chlordane which are recovered from the soil at 10-30 cm. Pesticide losses by surface runoff and leaching are interesting from the standpoints of pollution, effects on nontarget species, and reduced effectiveness of the pesticide at the target site (Baldwin et al., 1975). AG chlordane movement due to runoff was small, as has generally been reported for other pesticides (Bradley et al., 1972; Edwards and Glass, 1971; Caro and Taylor, 1971). On the other hand, data in Table III show that considerable movement of the α and γ isomers through the soil occurred. Additional soil samples were taken at 30-60- and 60-90-cm depths. Residue data obtained (0.012 ppm of α isomer and 0.003 ppm of γ isomer in the 30-60-cm layer from plots treated with 6 kg of AI/ha) show that α and γ isomers were, above all, confined to the 0-30-cm zone of soil. These are very important findings as they suggest that AG chlordane was retained largely in the target area. Water taken from underdrainage at the depth of 100 cm

is not contaminated (0.002 ppm for α-chlordane and 0.001 ppm for γ-chlordane).

Greater concentrations of α and γ isomers were encountered in the 0-14-cm soil layer in an earlier study (Tafuri et al., 1973-1974). The lower surface concentrations in the present study can be attributed to greater downward displacement due to greater total rainfall (384.0 mm as against 122.1 mm).

If the residues of α- and γ-chlordane observed at the two different depths (0-10 and 10-30 cm) are considered by mathematical calculations to be still all ideally present in the upper 0-10-cm layer of the soil (Table IV), statistical analysis demonstrates that there are highly significant differences in the concentrations of α-chlordane residues in soil at various sampling times, while the differences for γ isomer are not significant. As most of the γ-chlordane originally applied to the soil still persists after 169 days, it becomes clear that γ-chlordane is the more stable of the two isomers. When the two application rates of AG chlordane are compared, the residue amounts are significantly different (for α-chlordane at $p = 0.01$; for γ-chlordane at $p = 0.05$); thus, residue concentrations are influenced by different application doses. The interaction
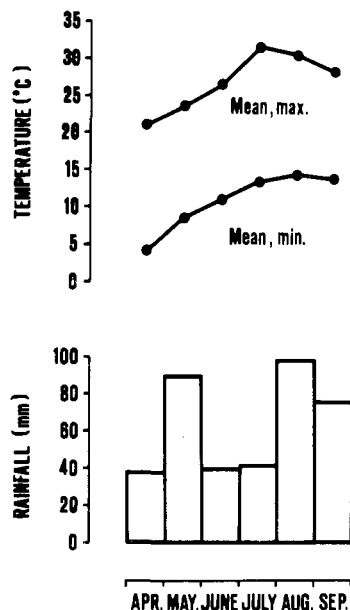
Figure 1. Environmental temperature and rainfall plotted against time after incorporation of the insecticide.

sampling times × application rates are not significant for either isomer; the residue decline in soil at two different application rates has a parallel trend.

The observed residues of α- and γ-chlordane, as determined by analysis of the alfalfa taken as cuts, unwashed and after washing, at the first and the second cuttings, are shown in Tables V and VI. The residue contents of α and γ isomers are not significantly different when the two application rates of AG chlordane are considered, but they are significantly different when compared with residue concentrations in the control alfalfa. The difference between the quantities of α- and γ-chlordane recovered in the analysis from washed and unwashed alfalfa would seem to suggest that an appreciable part is present on the plant surface, possibly in consequence of contamination by particles of treated soil. However, surface contamination is considerably less than the quantity which penetrates into the plant tissues, although the total amount recovered from washed plants is relatively small. Residues of chlordane isomers in alfalfa, sown in rotation after treated maize and wheat, were approximately of the same magnitude (Tafuri et al., 1974). α-Chlordane concen-

trations do not show significant differences between the first and second cuttings, while for γ-chlordane the mean difference is significant even if small. There does not seem to be any interaction between application rates, cuttings, and unwashed and washed alfalfa.

Photo-*cis*-chlordane residues in alfalfa were always less than the sensitivity limit (0.001 ppm). The average total residues of oxychlordane, found only in alfalfa at the second cutting, were 0.001 and 0.002 ppm following doses of 3 and 6 kg of AI/ha. Occurrence of greater amounts of photo-*cis*-chlordane and oxychlordane has been reported by others in a continental, semidesert climate (Wilson and Oloffs, 1973).

ACKNOWLEDGMENT

LITERATURE CITED

Baldwin, F. L., Santelmann, P. W., Davidson, J. M., *Weed Sci.* **23**(4), 285 (1975).
Bradley, J. R., Jr., Sheets, T. J., Jackson, M. D., *J. Environ. Qual.* **1**, 102 (1972).
Caro, J. H., Taylor, A. W., *J. Agric. Food Chem.* **19**, 379 (1971).
Dorough, H. W., Hemken, R. W., *Bull. Environ. Contam. Toxicol.* **10**, 208 (1973).
Edwards, W., Glass, B. L., *Bull. Environ. Contam. Toxicol.* **6**, 81 (1971).
Furness, W., *Proc. Br. Insectic. Fungic. Conf., 6th*, 541 (1971).
Gay, L., Cerny, M., *Mitt. Geb. Lebensmittelunters. Hyg.* **66**(2), 225 (1975).
Kovacs, A. I., Maini, P., *Proc. Int. Velsicol Symp., 6th* (1972).
Kovacs, A. I., Maini, P., Furness, W., *Proc. Br. Insectic. Fungic. Conf., 7th*, 649 (1973).
L'Hotellier, M., *Proc. Int. Velsicol Symp., 7th* (1973).
Martin, H., "Pesticide Manual", 4th ed, British Crop Protection Council, Oxford, 1974, p 95.
Melnikov, N. N., *Residue Rev.* **36**, 42 (1971).
Po-Yung, L., Metcalf, R. L., Hirwe, A. S., Williams, J. W., *J. Agric. Food Chem.* **23**(5), 967 (1975).
Tafuri, F., Businelli, M., Scarponi, L., Marucchini, C., *Agrochimica* **18**(1-2), 102 (1973-1974).
Tafuri, F., Businelli, M., Scarponi, L., Marucchini, C., *Ann. Fac. Agrar. Univ. Perugia* **29**, 219 (1974).
Wilson, D. M., Oloffs, P. C., *Bull. Environ. Contam. Toxicol.* **9**(6), 337 (1973).