Residues in Alfalfa Following Soil Treatment with High Purity Chlordane (Velsicol HCS-3260)

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INTRODUCTION

Oxychlordane, 1,2-dichlorochlordene epoxide (Fig. 1, III), is a recently described metabolite of chlordane (Fig. 1, I) occurring in some animals fed on chlordane or chlordane-treated forage (POLEN 1971). It has been found as a contaminant in milk and cheese from cows fed on alfalfa previously treated with technical chlordane (BOYD 1970, 1971; LAWRENCE et al. 1970). All reports have indicated that oxychlordane is formed only in animals and does not appear as a terminal residue in soil or crops (POLEN 1971). According to STREET and BLAU (1972), α- and γ-chlordane give rise to oxychlordane in animals via the intermediate 1,2-dichlorochlordene (Fig. 1, II).

Photo-cis-chlordane (Fig. 2, V) is a photoisomer of α-chlordane (Fig. 2, IV) similar in structure to photodieldrin. Sunlight or short wave U-V light produces a "half-cage" compound through hydrogen migration and carbon-carbon bond formation (BENSON et al. 1971). In contrast, γ-(trans)-chlordane does not photolyze to a half-cage structure because of steric hindrances of the chlorine atoms on the cyclopentane ring.

We report that occurrence of 1,2-dichlorochlordene, oxychlordane, and photo-cis-chlordane as well as the parent chlordane compounds in alfalfa after treatment of the soil with Velsicol HCS-3260.

MATERIALS AND METHODS

Insecticide and Standards

Soils were treated with high purity chlordane, recently developed by the Velsicol Chemical Corp., identified as HCS-3260. Its active ingredients consist of 95% or more of α-(=cis-) and γ-(=trans-) chlordane (VELSICOL CHEMICAL CORP. 1970). The ratio of cis-/trans- is appro. 3/1. Analytical reference

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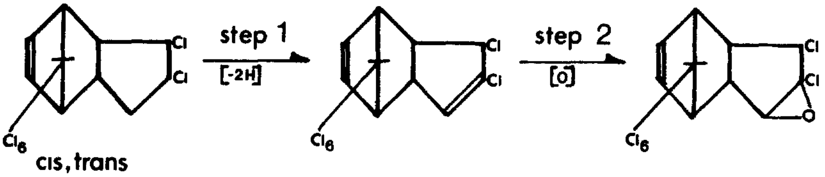
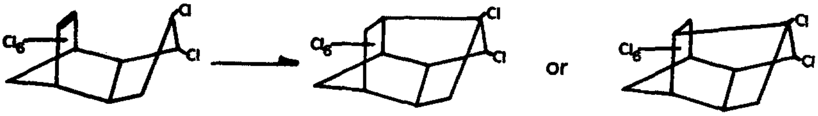
standards for the α- and γ- isomers, oxychlordane, and 1,2-dichlorochlordene were kindly supplied by the Velsicol Chemical Corp., photo-cis-chlordane by the Canada Department of Agriculture, Analytical Services Section, Ottawa.



I II III

Figure 1. Metabolism of chlordane (I) to 1,2-dichlorochlordene (II) and oxychlordane (III).



IV V

Figure 2. cis-chlordane (IV) and its photoisomers (V) (from Benson et al. (1)).

Treatment

The alfalfa (Lahontan variety) was grown at the Canada Department of Agriculture Research Station at Summerland, British Columbia. Six plots (20 x 20 ft. with 20 ft. buffer spaces) were staked and the alfalfa within each plot was cut to ground level with a lawn mower and raked clean. Appropriate amounts of HCS-3260 EC were diluted with water and applied to the soil with a HAWS 2-gallon watering can in June, 1971. Four gallons of emulsion were used to cover each plot in one direction; a further 4 gallons were then applied in the opposite direction. The treatment rates were 5 or 10 lbs a.i./acre, in two replicates. Two plots were left untreated as controls. All the plots were thoroughly watered after treatment.

Sampling

Alfalfa was sampled 2 months after soil treatment and cut to about 4 inches 3 months after treatment. The new growth was sampled at 4 months (i.e., 1 months after cutting) and again 1 year after treatment. The samples were washed under running water, dried, and frozen at -20°C until extraction.

Extraction and Cleanup

Subsamples from each plot were macerated and 100 g duplicate aliquots extracted in 400 ml of hexane:acetone 1:1 (v/v) for 30 minutes in a Lourdes homogenizer. After 20 minutes, 25 g of anhydrous Na_2SO_4 were added. The extracts were decanted through glass wool into separatory funnels and the acetone was removed by washing three times with 200 ml 2% aqueous Na_2SO_4. Twenty ml of the crude hexane extract were concentrated to 2 ml and cleaned on 6 g 100-200 mesh Florisil. Forty ml of hexane followed by 40 ml of benzene:hexane 5:1 (v/v) were used to elute the compounds from the columns. The eluates were taken to dryness in a flash evaporator and the residues taken up with 5 ml hexane.

GLC Analysis

GLC analysis was in a Microtek MT-220 equipped with dual ^{63}Ni electron capture detectors. Three glass columns were used (183 cm x 0.64 cm O.D.), packed with mixtures of: (I) 2% OV 1 + 6% OV 210; (II) 2% SE 30 + 6% QF 1; and (III) 1.50% OV 17 + 1.95% OV 210 on Chromosorb W "H.P.", 80/100 mesh. N_2 was used as carrier gas at 80 ml/min. Common flow to one detector was accomplished by means of a combiner for columns (II) and (III). Purge gas on the EC detector for column (I) was 20 ml/min. There was no purge gas on the EC detector for columns (II) and (III). Temperatures were: injector 220°C, oven 190°C, detectors 300°C. Standard curves were prepared before and after each analysis.

Isolation and Confirmation of Oxychlordane

Column Chromatography: Analyses of the extracts by GLC indicated the presence of α-, γ-, oxy-, and photo-cis-chlordane. On 6 g Florisil, using 0.2 µg of analytical reference standards of the above compounds, α-, γ-, and photo-cis-chlordane were separated from oxychlordane by eluting with 60 ml of pet. ether:hexane 2:3 (v/v). Oxychlordane was then eluted using 40 ml of benzene:hexane 5:1 as described. For the field samples, 20 ml of alfalfa extract were concentrated, and oxychlordane was separated from the other metabolites in the same way.

Chemical Derivatization: Reductive dechlorination using aqueous chromous chloride ($CrCl_2$) solution as described by COCHRANE and CHAU (1970) was performed on 0.8 μg analytical reference oxychlordane and on approximately 0.3 μg oxychlordane isolated from the alfalfa extract by column chromatography.

Gas Chromatography: In addition to the GLC columns already described, a more polar 7% OV 225 liquid phase on 60/80 mesh Gas Chrom Q was also used for confirmation. GLC conditions for this column were as described.

p-Value: For hexane-acetonitrile partitioning of both cleaned-up alfalfa extracts and analytical reference oxychlordane in hexane, we used the method described by BEROZA et al. (1969).

Recovery Tests

Macerated alfalfa tissue from the control plots was fortified with known amounts of analytical standards of α-, γ-, oxy-, and photo-cis-chlordane. Recoveries from duplicate fortified samples were between 94 and 109%.

RESULTS AND DISCUSSION

Figures 3 and 4 show the chlordane residues in alfalfa at the two application rates and three sampling intervals. The initial growth of alfalfa, from 24 to 30 inches high, was sampled 2 months after treatment. It had somewhat higher residues than the new growth sampled 4 months after treatment. Samples taken 1 year after treatment had a marked reduction in residues. In contrast, the concentrations of α- and γ-chlordane in the soil did not decline; similar amounts were detected after 4 months and 1 year (WILSON and OLOFFS unpublished).

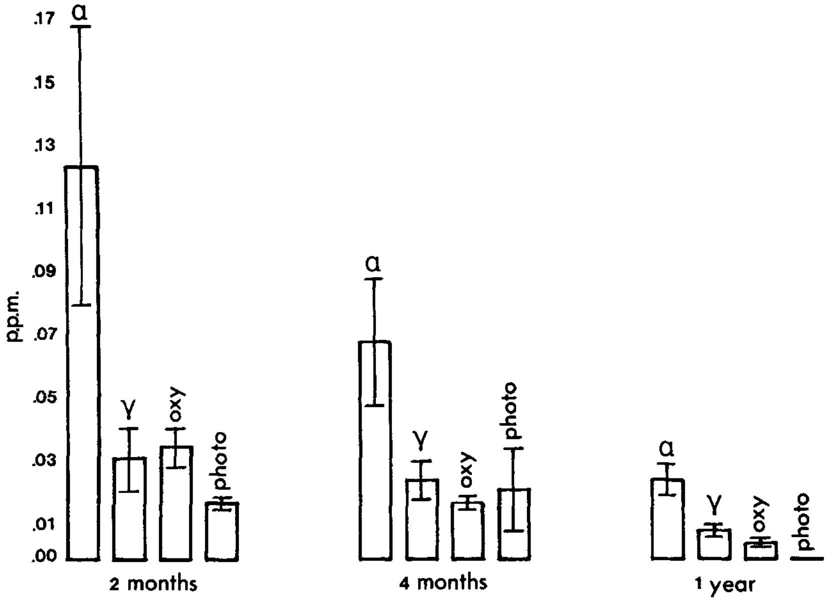


Figure 3. Chlordane residues in alfalfa at intervals following soil treatment of 5 lbs a.i./acre (mean and S.D. in p.p.m. of fresh weight).

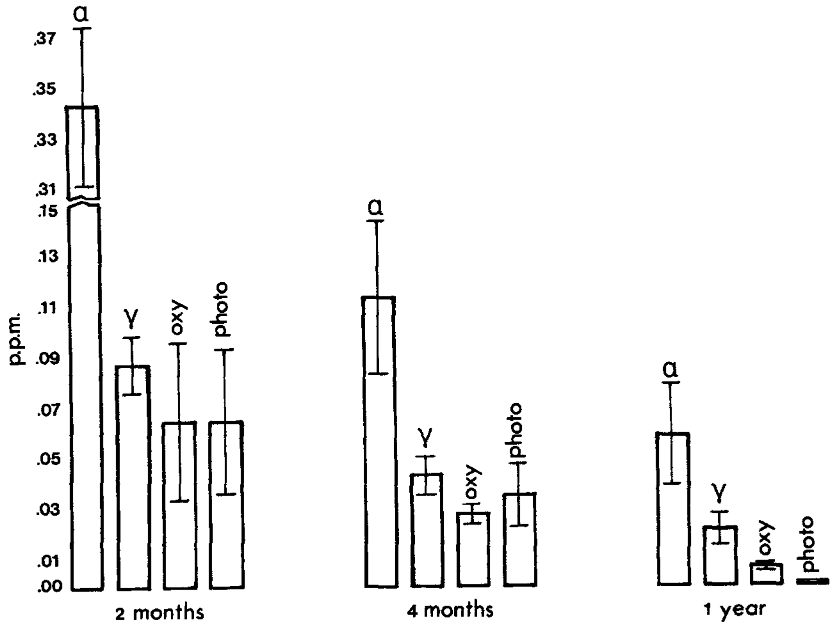


Figure 4. Chlordane residues in alfalfa at intervals following soil treatment of 10 lbs a.i./acre (mean and S.D. in p.p.m. of fresh weight).

Oxychlordane comprised from 9 to 13% of the total residues (α- + γ- + oxy- + photo-cis-chlordane) in the alfalfa from the plots treated at 10 lbs/acre, and 13 to 17% in that from the plots treated at 5 lbs/acre. It appears to be endogenous since it could not be detected in the soils. The intermediary compound, 1,2-dichlorochlordene, was detected at 0.011 ppm in the alfalfa from the 10-lb plots, and in trace quantities in the other.

Since these results seem to be the first indication that oxychlordane can appear as a residue in plants, further confirmation of identity was very important, especially in view of previous misidentification of oxychlordane for heptachlor epoxide (COCHRANE 1971). Our columns (I) and (II) gave good separation of, and sensitivity to, those compounds. The separation of α-, γ-, and oxychlordane and heptachlor epoxide on our column (III) has recently been described (CONDER et al. 1972). The more polar OV 225 column also clearly separated oxychlordane from heptachlor epoxide but did not resolve α- and γ-chlordane. GLC analysis of alfalfa samples from the control plots showed no peaks interfering with any of the above-mentioned compounds. p-values for standard and extracted oxychlordane were 0.44 and 0.45 respectively. Standard and extracted oxychlordanes were monodechlorinated with $CrCl_2$ by attack on the gemdichloro group present in the hexachlorocyclopentene moiety to yield the sym- and anti-heptachloro- derivatives (COCHRANE and CHAU 1971), and then confirmed by GLC.

Photo-cis-chlordane[1/] accounted for 9 to 16% of the total residues in the alfalfa from both 5- and 10-lb plots. It was also found in the soil (max. 0.15 ppm) for four months following the treatment. The occurrence of this photo-isomer was probably due to a combination of the method of application of HCS-3260, its composition, and the continental, semi-desert climate of the area at that time (Table 1).

[1/] Also identified by the Analytical Services Section, Canada Dept. of Agriculture, Ottawa.

TABLE 1

Climatic Conditions for Sampling Period
June to October 1971.*

	Bright sunlight (hours)	Precipitation (inches)	Average air temp. (^{o}F)
June	233.9	2.06	59.5
July	357.9	0.47	69.8
Aug.	351.8	1.19	74.0
Sept.	182.5	0.76	56.6
Oct.	156.1	0.54	46.3

* Compiled from CHAPMAN, F.M. "Weather Observations for 1971", Canada Dept. of Agriculture, Research Station, Summerland, B.C. (1972).

SUMMARY

Residues of chlordane were determined in alfalfa up to one year after application of Velsicol HCS-3260 to soil at 5 and 10 lbs active ingredient per acre. The major residues were cis-, trans-, photo-cis-, and oxychlordane. The latter two accounted for a maximum of 16% and 17%, respectively, during the first four months after treatment. The identity of oxychlordane was confirmed by four different GLC columns, chemical derivatization, p-value determination, and column chromatography.

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